Technical Resource and Product Guide

Essential Biochemicals for Research

The foundation for publication quality results

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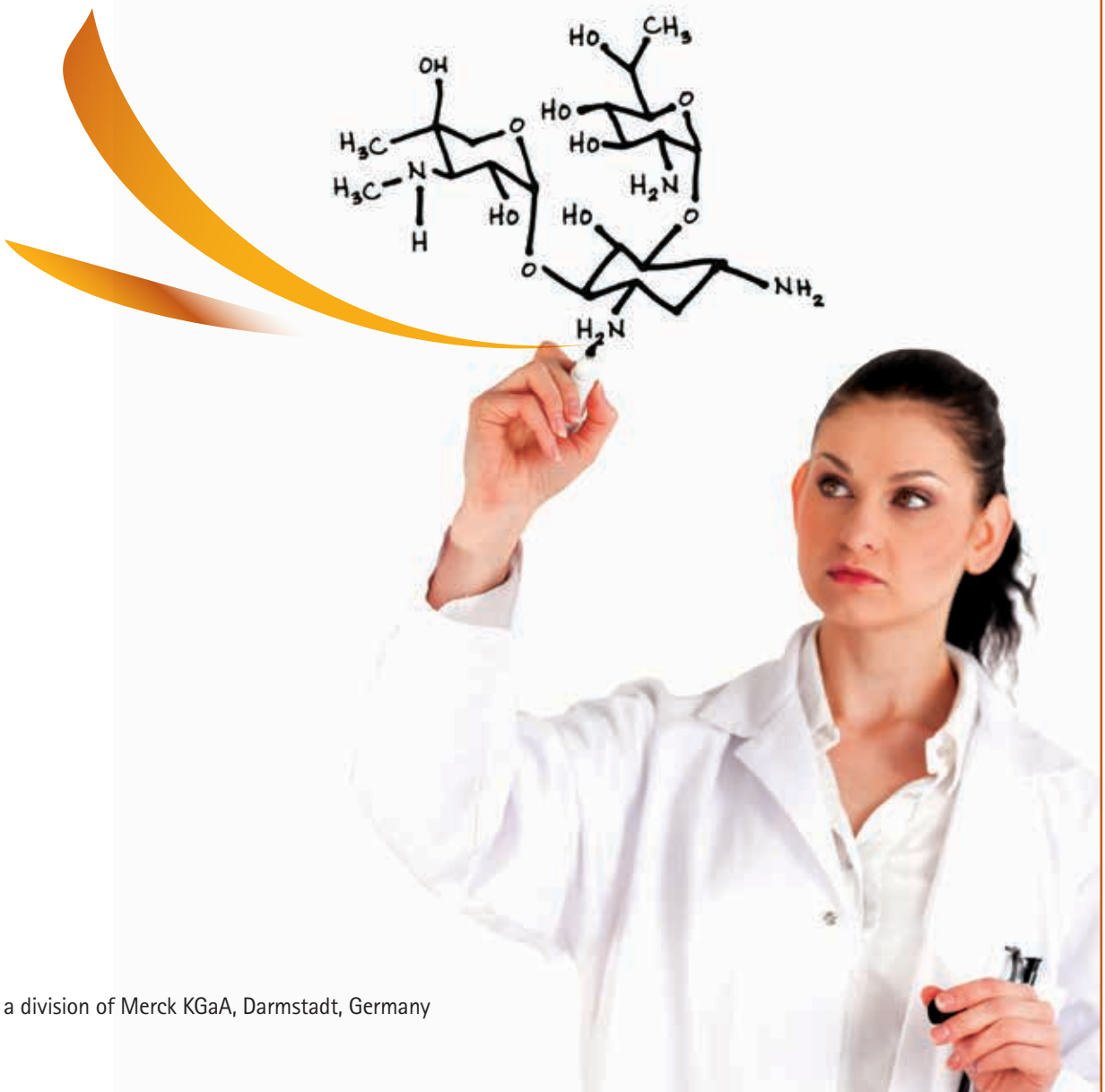

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany

Research Essential Biochemicals from EMD Millipore.
The foundation for publication quality results.

Dear Customer,

This guide is a resource for scientists for the preparation and use of research essential biochemicals, such as antibiotics, buffers, detergents, dyes, stains, and substrates, which are indispensable for any life science research laboratory. You will find this guide to be a useful resource, whether you are just beginning your research or you are training the new researchers in your laboratory.

Specific technical areas and workflows, such as molecular biology, cellular analysis, chromatographic separation, and protein detection, require certain specific biochemicals with particular characteristics. Similarly, certain research focus areas also have specific requirements. Most of our reagents are discussed in depth on our website and in technical literature devoted to technical applications and research areas.

The research essential biochemicals discussed in this guide have utility and importance that cover multiple techniques and cut across multiple research fields, including cancer and apoptosis, cell signaling, neuroscience, cell health and toxicity, metabolism, cell structure, epigenetics, inflammation and immunology, and stem cell research.

As your life science partner, EMD Millipore recognizes that reproducible, interpretable, and ultimately publishable results are only possible with the use of high quality reagents in every experiment. Hence, we work hard to provide you with well-documented, well-characterized, and highly cited reagents.

We are frequently asked for advice on protocols and reagents. This practical resource represents our commitment to provide valuable information and exceptional service to facilitate the success of your research.

For more information and any specialized advice, please contact our technical support team and give us an opportunity to serve you.

EMD Millipore

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Antibiotics

1

Antibiotics are naturally occurring chemicals secreted by one microorganism to defend against attack from other microorganisms, including bacteria, viruses, fungi, protozoans, and helminths. Since the 1940s, antibiotics have been exploited for therapeutic and research purposes. The most common research use of antibiotics is for selection and screening of genetically modified prokaryotes and eukaryotes. To choose the most appropriate antibiotic and selection marker system for an experiment, it is useful to understand antibiotics' mechanism of action.

Antibiotic Action on Bacteria

Many metabolic activities of the bacterial cell differ from those in mammalian cells. These differences can be exploited in the development of antibiotic agents. Antibiotics can either kill the bacteria (bactericidal) or prevent their growth (bacteriostatic). Antibiotics act on bacteria in one of the following ways:

A. Inhibition of cell wall synthesis
B. Inhibition of protein synthesis
C. Inhibition of nucleic acid synthesis
D. Anti-metabolic activity or competitive antagonism

A. Inhibition of Cell Wall Synthesis

Bacterial cell walls contain repeating units of peptidoglycans, which are not found in mammalian cells. Hence, the bacterial cell wall presents an ideal target for antibiotic development and therapy. The biosynthesis of peptidoglycan occurs in three stages (left column), and certain antibiotics (right column) can specifically inhibit each of these stages:

Stage of cell wall synthesis	Antibiotic and mechanism
N-acetylglucosamine and N-acetyl-muramyl-pentapeptide are synthesized in the cytoplasm as UDP derivatives.	**D-cycloserine**, a structural analog of D-alanine, binds to the substrate-binding site of two enzymes involved in the synthesis, preventing D-alanine from being incorporated into the N-acetyl-muramylpeptide.
Transfer of N-acetyl-muramylpeptide from UDP to a carrier lipid, followed by a subsequent modification to form a complete nascent peptidoglycan subunit.	**Bacitracin** inhibits lipid phosphatase activity.
Peptidoglycans grow in size.	**Vancomycin**: binds D-alanyl-D-alanine peptide termini of the nascent peptidoglycan-lipid carrier and inhibits the enzyme transglycosylase. **β-lactam antibiotics (Penicillins, Carbenicillin, Cephalosporins):** structural analogs of the D-alanyl-D-alanine end of the peptidoglycan strand, these antibiotics bind the substrate-binding site of enzymes that act upon the D-alanyl-D-alanine end.

B. Inhibition of Protein Synthesis

An antibiotic that inhibits either transcription or translation will inhibit protein synthesis. It is possible to selectively target bacterial transcription because bacterial ribosomes differ significantly from those of higher organisms.

Steps of protein synthesis	Antibiotic and mechanism
Transcription (RNA synthesis)	**Actinomycin D**: distorts DNA by binding to guanine and blocks transcription **Rifampicin**: inhibits DNA-dependent RNA polymerase
Translation (initiation, elongation, and termination of the peptide chain)	**Tetracyclines**: block binding of aminoacyl-tRNA to the A site on the ribosome **Chloramphenicol**: blocks bacterial translation by binding the 70S ribosome; blocks mitochondrial protein synthesis in higher organisms because mitochondria also have 70S ribosomes. **Aminoglycosides, such as kanamycin**: irreversibly inhibit protein synthesis following their binding to the 30S subunit of the bacterial ribosome. Disruption in protein synthesis by aminoglycosides occurs by three different modes: (a) by interfering with the initiation complex; (b) by inducing a misreading of the genetic code; and (c) by promoting the breakup of polysomes into monosomes that are incapable of participating in protein synthesis. **Macrolides**: bacteriostatic for most bacteria, but bacteriocidal for a few Gram-positive bacteria. They inhibit protein synthesis by binding to the 50S ribosomal subunit, which blocks the elongation of the protein by peptidyl transferase.

C. Inhibition of Nucleic Acid Synthesis

Most inhibitors of DNA replication bind to DNA and are considered to be too toxic for any clinical use, but they are useful research tools.

Aspects of nucleic acid synthesis	Antibiotic and mechanism
DNA replication, all steps	**Actinomycin D, daunorubicin, doxorubicin, and epirubicin**: all inhibit DNA replication by intercalating DNA; are used as antitumor agents
DNA uncoiling and coiling in prokaryotes	**Nalidixic acid**: binds and inhibits DNA gyrase **Rifampicin**: a semi-synthetic derivative of rifamycin that is active against Gram-positive bacteria and a few Gram-negative bacteria. Rifampicin acts specifically on eubacterial RNA polymerase. It binds to the β-subunit of the polymerase and blocks the entry of the first nucleotide, which is essential for polymerase activation. It is found to be highly effective against the Mycobacterium that causes tuberculosis.
DNA uncoiling and coiling in prokaryotes AND mammalian cells	**Quinolones**: block the A subunit of DNA gyrase (a topoisomerase) and induce the formation of a relaxation complex analog. **Ciprofloxacin**: potent inhibitor of DNA gyrase.

D. Anti-metabolic Activity or Competitive Antagonism

Antibiotics with structural similarity to essential metabolites and bacterial growth factors inhibit bacterial metabolic pathways via competitive antagonism.

Metabolic pathway	Antibiotic and mechanism
Tetrahydrofolate (THF) synthesis	**Sulfonamides**: structurally similar to para-aminobenzoic acid (PABA), the substrate for the first enzyme in the THF pathway. Sulfonamide-sensitive bacteria include *Streptococcus pneumoniae*, β-hemolytic *Streptococci*, *E. coli*, and many others. Sulfonamides can also cross the blood-brain barrier and are useful in the treatment of meningococcal meningitis. **Trimethoprim**: structurally similar to dihydrofolate (DHF) and competitively inhibits the second step in THF synthesis that is mediated by the DHF reductase. Since higher animals do not synthesize their own folic acid, they are not affected by these antimicrobial agents.
Mycolic acid synthesis in Mycobacterium	**Isoniazid**: an analog of pyridoxine (vitamin B6), inhibits acyl carrier protein AcpM and Kas A in the mycolic acid synthesis pathway. Isoniazid is activated by a mycobacterial peroxidase enzyme.
DNA uncoiling and coiling in prokaryotes AND mammalian cells	**Quinolones**: block the A subunit of DNA gyrase (a topoisomerase) and induce the formation of a relaxation complex analog. **Ciprofloxacin**: potent inhibitor of DNA gyrase

Other Mechanisms

Certain cationic aminoglycosides, such as gentamicin, perturb the packing order of lipids and destabilize bilayered membranes, resulting in cell lysis.

Antibiotics Commonly Used for Mammalian Cells

Hygromycin B

Hygromycin B displays broad spectrum activity against prokaryotes and eukaryotes by strongly inhibiting protein synthesis. Like other aminoglycoside antibiotics, hygromycin B distorts the structure of the ribosome, inducing misreading of tRNAs. Also, in the presence of hygromycin B, mRNA is often mistranslocated.

Biological applications:

- Hygromycin B-resistance gene is useful in identification and selection of recombinant clones in a variety of cell types. A gene from *E. coli* encoding resistance to Hygromycin B can be isolated and cloned by recombinant DNA techniques into bacteria, yeast, or mammalian cells, making them Hygromycin B-resistant.
- Hygromycin B can be used as a selection agent for the transfer of a drug-resistant gene between prokaryotes and eukaryotes.
- Hygromycin B resistance may be used in construction of retroviral cloning vectors.
- Hygromycin B may be used as an antiviral drug in the treatment of murine coronaviral hepatitis.
- Hygromycin B may be used as an inhibitor of RNA translation.

Blasticidin S

Blasticidin S inhibits protein synthesis in both prokaryotes and eukaryotes by inhibiting peptide bond formation by the ribosome.

Puromycin

Puromycin specifically inhibits peptidyl transfer on both prokaryotic and eukaryotic ribosomes, inhibiting growth of gram-positive bacteria, animal, and insect cells. Fungi and gram-negative bacteria are relatively impermeable to puromycin and are therefore resistant.

Neomycin

An aminoglycoside, neomycin inhibits protein synthesis by binding to the ribosome, interfering with translation intitiation, inducing misreading, and promoting breakup of ribosomal polysomes.

G418 Sulfate

An aminoglycoside related to gentamycin. It inhibits prokaryotic and eukaryotic protein synthesis. G418 Sulfate is widely used in the selection of eukaryotic expression vectors carrying the bacterial *neoR/kanR* genes.

Bleomycin

Bleomycin causes cell death by inducing double strand breaks in DNA.

Resistance to Antibiotics

Bacteria are generally known to develop or acquire resistance to antibiotics over time and render them ineffective as therapeutic agents. There are four major ways by which bacteria become resistant to antibiotics: alteration in cell wall, production of antibiotic-degrading enzymes, change in antibiotic target, and synthesis of antibiotic efflux pumps or transporters (see Figure 1.1).

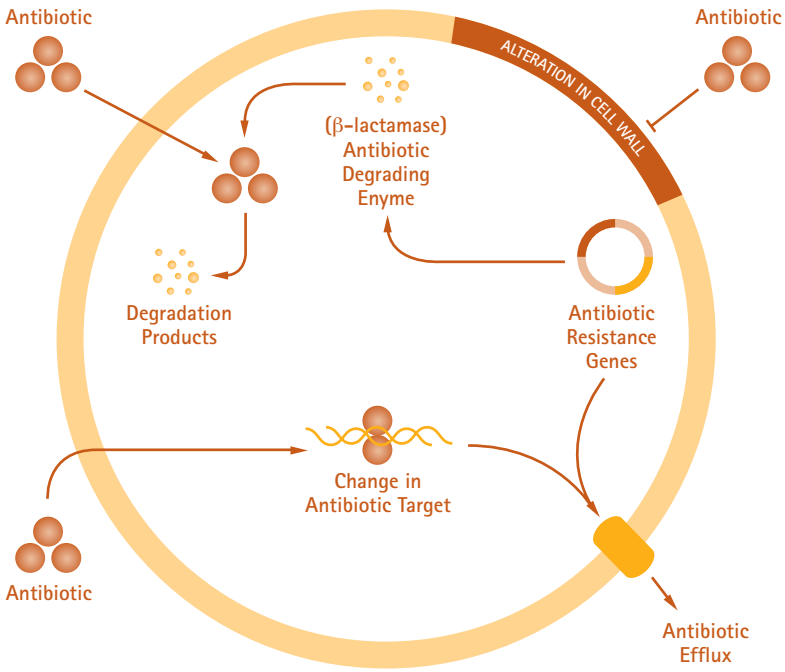


Figure 1.1
Antibiotic resistance mechanisms

Natural Resistance:

Bacteria may be naturally resistant to certain antibiotics and have gene products that protect them from their own antibiotics. For example, Gram-positive bacteria are less susceptible to polymyxins than Gram-negative bacteria; Gram-negative bacteria have an outer membrane that establishes a permeability barrier against the antibiotic; some bacteria may lack a transport system for the antibiotic and others may synthesize "efflux pumps" in their plasma membrane through which they may remove antibiotics.

Acquired Resistance

Bacteria may develop resistance to antibiotics to which they were previously susceptible. These changes result from mutations and selections or exchange of genes between different strains and species. For example, synthesis of penicillinase (or other β-lactamases) provides protection from the β-lactam antibiotics.

Markers

A marker gene is used in molecular biology to determine if a nucleic acid sequence on the same vector has been successfully inserted into an organism's DNA.
There are two types of marker genes:

Selectable marker: protects the organism from a selective agent that would normally kill it or prevent its growth. A selectable marker is a gene introduced into a cell, especially a bacterium or to cells in culture, which confers a trait suitable for artificial selection. Selectable markers can indicate the success of transfection or other procedure meant to introduce foreign DNA into a cell. Selectable markers are often antibiotic resistance genes; bacteria that have been subjected to a procedure to introduce foreign DNA are grown on a medium containing an antibiotic, and those bacterial colonies that can grow have successfully taken up and expressed the introduced genetic material. In most applications, only one in a several billion cells will take up DNA. Rather than checking every single cell, scientists use a selective agent to kill all cells that do not contain the foreign DNA, leaving only the desired ones.

Antibiotics are the most common selective agents. In bacteria, antibiotics are used almost exclusively. In plants, antibiotics that kill the chloroplast are often used as well, although tolerance to salts and growth-inhibiting hormones is becoming more popular. In mammals, resistance to antibiotics that would kill the mitochondria is used as a selectable marker. Examples of selectable markers include:

- *ampR* – ampicillin resistance gene
- *chlR* –chloramphenicol resistance gene
- *neoR* - neomycin resistance gene, confers antibiotic resistance to G418 sulfate and neomycin

Screenable marker: allows the researcher to visually distinguish between wanted and unwanted cells. Screenable markers are not typically antibiotic resistance genes. Examples include:

- β-galactosidase – can convert certain substrates to blue-colored products, other substrates are fluorogenic
- β-glucuronidase – can stain cells blue, used mostly for plant cells

How to Generate a Stably Transfected Mammalian Cell Line Using Antibiotic Selection

Different cell lines exhibit different sensitivities to antibiotics. The sensitivity of the cell population to antibiotics depends on several factors. These include the rate of division of the cell, the baseline level of toxicity and whether or not the parent (untransfected) cell line has become resistant. Many commonly used cell lines have become resistant to multiple antibiotics, usually by acquiring a resistance plasmid. In addition, they are highly susceptible to mutations that may lead to resistance.

Using an antibiotic concentration that is too high may result in nonspecific cytotoxicity, potentially killing desirably transfected cells. Using an antibiotic concentration that is too low may result in cells being exposed to selective media for too long; the longer cells are exposed to antibiotics, the more likely that resistant populations will emerge that may not contain the gene of interest.

Therefore, prior to transfection, it is important to determine the antibiotic concentration that effectively kills untransfected cells ("kill curve").

1. Select at least 6 concentrations of antibiotic to apply to cells (example: 0, 50, 100, 200, 400, 800, 1000 µg/mL)
2. Seed culture dishes (or wells of a plate) with identical number of cells per concentration point at 25% confluency in appropriate culture medium with no antibiotic.
3. After 24 hours, replace the medium with medium containing the desired concentrations of antibiotic.
4. Replace the medium every 3-4 days.
5. Count the number of viable cells each time.
6. Choose the antibiotic concentration in which all the cells are killed in 7-10 days.

Preparing Antibiotics Before Application

Antibiotic solutions are often sterile filtered before use so as to minimize the introduction of mycoplasma or other contaminants that can have a negative effect on the experiment. EMD Millipore's Millex® syringe filters set the standard for reliable small volume sterile filtration. Millex® filters can be used to sterilize tissue culture media and additives, protein solutions, virus suspensions, DNA, and other aqueous solutions, and are available with three membranes: Millipore Express® (PES) membrane, mixed cellulose esters (MCE), and Durapore (PVDF). Choose the filters that work best for your application:
Millex®-GP filters feature Millipore Express® (PES) membrane, which provides a unique combination of speed and six times less protein binding than competitive PES membranes. Millex®-GP filters are recommended for sterile filtering protein solutions, tissue culture media, buffers and additives.

Millex® filters with Durapore® (PVDF) membrane are the lowest protein-binding syringe filters available. Use them to filter protein solutions. The 0.2 µm filters are sterilizing grade. Larger pore sizes are for clarification and prefiltration.

Millex®-MF filters feature mixed cellulose esters (MCE) membrane, which provides reliable, general purpose filtration of water or other aqueous solutions. The 0.2 µm filters are sterilizing grade. Larger pore sizes are for clarification and prefiltration.

Sterilize antibiotic solutions using Millex® syringe filters

- 0.22 µm pore size for sterilization and 0.10 µm pore size for filtering out mycoplasma
- Variety of membrane types to ensure maximum chemical compatibility, low binding, and fast filtration
- Gamma-irradiated or ethylene oxide-treated filters individually packaged, ensure worry-free preparation of selective cell culture media

Units with fast flow and low protein binding Millipore Express (PES) membrane

Description	Color Code	Pore Size (µm)	Qty/Pk	Catalogue No.
Millex®-GP Filter Unit	Green	0.22	1000	SLGP033RK
Millex®-GP Filter Unit	Green	0.22	250	SLGP033RB
Millex®-GP Filter Unit	Green	0.22	50	SLGP033RS
Millex®-HP Filter Unit	Green	0.45	50	SLHP033RS
Millex®-HP Filter Unit	Green	0.45	250	SLHP033RB

Units with MF–Millipore (mixed cellulose esters) membrane

Description	Color Code	Pore Size (µm)	Qty/Pk	Catalogue No.
Millex-GS Filter Unit	Blue	0.22	50	SLGS033SS
Millex-GS Filter Unit	Blue	0.22	250	SLGS033SB
Millex-HA Filter Unit	Blue	0.45	50	SLHA033SS
Millex-HA Filter Unit	Blue	0.45	250	SLHA033SB
Millex-AA Filter Unit	Blue	0.8	50	SLAA033SS
Millex-AA Filter Unit	Blue	0.8	250	SLAA033SB

Units with very low protein binding Durapore (PVDF) membrane

Description	Color Code	Pore Size (µm)	Qty/Pk	Catalogue No.
Millex-VV Filter Unit	Yellow	0.1	50	SLVV033RS
Millex-GV Filter Unit	Yellow	0.22	50	SLGV033RS
Millex-GV Filter Unit	Yellow	0.22	250	SLGV033RB
Millex-GV Filter Unit	Yellow	0.22	1000	SLGV033RK
Millex-HV Filter Unit	Yellow	0.45	50	SLHV033RS
Millex-HV Filter Unit	Yellow	0.45	250	SLHV033RB
Millex-HV Filter Unit	Yellow	0.45	1000	SLHV033RK

EMD Millipore Calbiochem® Brand Antibiotics and Selection Agents

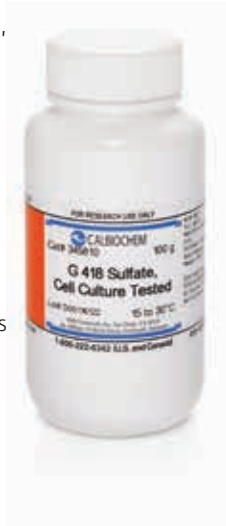
Advantages

- Cell culture tested
- Offer superior potency
- Have more than 10,000 citations since year 2000
- Manufactured in a highly controlled environment, ensuring the utmost in purity and quality

For complete product listings and comprehensive guides to antibiotic selection, please visit our online antibiotics resource at: **www.emdmillipore.com/antibiotics**

G418 Sulfate, Cell Culture Tested (Catalogue Nos. 345810, 345812)

G418 sulfate (G418) is an aminoglycoside related to gentamycin that inhibits prokaryotic and eukaryotic protein synthesis. Toxic to bacteria, yeast, protozoans, helminths, higher plant, and mammalian cells, G418 is widely used in the selection of eukaryotic expression vectors carrying the bacterial *neor/kanr* genes. The product of these genes, aminoglycoside 3′-phosphotransferase, inactivates G418, neomycin, and kanamycin by phosphorylation. Introduction of either of these genes into cells can confer resistance to G418, which enables cells to grow in media containing G418.



Hygromycin B, *Streptomyces* sp., Sterile-Filtered Solution in PBS, Cell Culture-Tested (Catalogue No. 400052)

Hygromycin is an unique aminoglycoside antibiotic that inhibits the growth of prokaryotic (bacteria) and eukaryotic microorganisms (yeasts) and mammalian cells. Inhibits protein synthesis at the translocation step on the 70S ribosome and causes misreading of the mRNA. *Hph*, a gene from *E. coli*, encodes resistance to hygromycin B and can be isolated and cloned by recombinant DNA techniques. This hygromycin B-resistance gene is particularly useful for identification or selection of recombinant clones in a variety of cell types.

Potency and Purity Comparison to G418 from other suppliers:

Supplier	Potency Specifications	Purity
EMD Millipore	≥730 µg/mg	≥98%
Supplier I	≥700 µg/mg	≥90%
Supplier S	≥450 µg/mg	Not specified

Key Products

Description	Catalogue No.
Blasticidin S, HCl, *Streptomyces griseochromogenes*	203350
Actinomycin D, 7-Amino-	129935
Puromycin, DiHCl. Cell Culture Tested	540411
Bleomycin Sulfate, *Streptomyces verticillus*	203401

For more key antibiotics from EMD Millipore, see page 62.

2 Buffers

Almost all biological processes are pH-dependent. Even a slight change in pH can result in metabolic acidosis or alkalosis, resulting in severe metabolic complications. The purpose of a buffer in a biological system is to maintain intracellular and extracellular pH within a very narrow range and resist changes in pH in the presence of internal and external influences. Our discussion of buffers and how they control hydrogen ion concentrations begins with a brief explanation of the role of water and equilibrium constants of weak acids and bases.

Chemistry and Properties of Buffers

Role of Water and Buffers in Biological Systems

All biological reactions occur in an aqueous medium, and all aspects of cell structure and function are adapted to the physical and chemical properties of water. Because water is such a polar solvent, it dissolves most salts, acids, and bases by hydrating and stabilizing dissociated anions and cations.

Biological systems usually contain weak acids and bases. Weak acids and bases do not completely dissociate in solution. They exist instead as an equilibrium mixture of undissociated and dissociated species. The equilibrium constant for ionization reactions is called the ionization constant or dissociation constant.

Buffers are aqueous systems that resist changes in pH when small amounts of acid or base are added. Buffer solutions are composed of a weak acid (the proton donor) and its conjugate base (the proton acceptor). Buffering results from two reversible reaction equilibria in a solution wherein the concentration of proton donor and its conjugate proton acceptor are equal. For example, in a buffer system when the concentrations of acetic acid (CH_3COOH) and acetate ions (CH_3COO^-) are equal, addition of small amounts of acid or base does not have any detectable influence on the pH. This point is commonly known as the isoelectric point. At this point there is no net charge and pH at this point is equal to pK_a.

$$pH = pK_a + \log \frac{[CH_3COO^-]}{[CH_3COOH]}$$

At isoelectric point $[CH_3COO^-] = [CH_3COOH]$; hence,

$$pH = pK_a$$

Henderson-Hasselbach Equation: pH and pK_a

The relationship between pH, pK_a, and the buffering action of any weak acid and its conjugate base is best explained by the Henderson-Hasselbalch equation. In biological experiments, $[H^+]$ varies from 10^{-1} M to about 10^{-10} M. Hence, for $[H^+]$ one can write the following equation:

$$pH = -\log [H^+]$$

Similarly pK_a can be defined as – log Ka. If the equilibrium expression is converted to – log, then

$$-\log [H^+] = -\log K_a - \log \frac{[CH_3COOH]}{[CH_3COO^-]}$$

and pH and pK_a substituted:

$$pH = pK_a - \log \frac{[CH_3COOH]}{[CH_3COO^-]}$$

or

$$pH = pK_a + \log \frac{[CH_3COO^-]}{[CH_3COOH]}$$

When the concentration of acetate ions equals the concentration of acetic acid, $\log [CH_3COO^-]/[CH_3COOH]$ approaches zero (the log of 1) and pH equals pK_a (the pK_a of acetic acid is 4.745). Acetic acid and acetate ion form an effective buffering system centered around pH 4.75. Generally, the pK_a of a weak acid or base indicates the pH of the center of the buffering region.

Determination of pK_a

pK_a values are generally determined by titration. A carefully calibrated, automated, recording titrator is used, the free acid of the material to be measured is titrated with a suitable base, and the titration curve is recorded. The pH of the solution is monitored as increasing quantities of base are added to the solution.

Table 2.1 pK_a values for commonly used biological buffers and buffer constituents

Description	M.W.	pK_a at 20 °C	Catalogue No.
BES*, ULTROL® Grade	213.2	7.15	391334
Bicine, ULTROL® Grade	163.2	8.35	391336
BIS-Tris, ULTROL® Grade	209.2	6.50	391335
BIS-Tris Propane, ULTROL® Grade	282.4	6.80	394111
Boric Acid, Molecular Biology Grade	61.8	9.24	203667
Cacodylic Acid	214.0	6.27	205541
CAPS, ULTROL® Grade	221.3	10.40	239782
CHES, ULTROL® Grade	207.3	9.50	239779
Glycine	75.1	2.34[1]	3570
Glycine, Molecular Biology Grade	75.1	2.34[1]	357002
Glycylglycine, Free Base	132.1	8.40	3630
HEPES, Free Acid, Molecular Biology Grade	238.3	7.55	391340
HEPES, Free Acid, ULTROL® Grade	238.3	7.55	391338
HEPES, Free Acid Solution	238.3	7.55	375368
HEPES, Sodium Salt, ULTROL® Grade	260.3	7.55	391333
Imidazole, ULTROL® Grade	68.1	7.00	4015
MES, Free Acid, ULTROL® Grade	195.2	6.15	475893
MES, Sodium Salt, ULTROL® Grade	217.2	6.15	475894
MOPS, Free Acid, ULTROL® Grade	209.3	7.20	475898
MOPS, Sodium Salt, ULTROL® Grade	231.2	7.20	475899
PIPES, Free Acid, ULTROL® Grade	302.4	6.80	528131
PIPES, Sodium Salt, ULTROL® Grade	335.3	6.80	528132
PIPPS	330.4	3.73[2]	528315
Potassium Phosphate, Dibasic, Trihydrate, Molecular Biology Grade	228.2	7.21[3]	529567
Potassium Phosphate, Monobasic, Molecular Biology Grade	136.1	7.21[3]	529568
Sodium Phosphate, Dibasic	142.0	7.21[3]	567550
Sodium Phosphate, Dibasic, Molecular Biology Grade	142.0	7.21[3]	567547
Sodium Phosphate, Monobasic	120.0	7.21[3]	567545
Sodium Phosphate, Monobasic, Monohydrate, Molecular Biology Grade	138.0	7.21[3]	567549
TAPS, ULTROL® Grade	243.2	8.40	394675
TES, Free Acid, ULTROL® Grade	229.3	7.50	39465
Tricine, ULTROL® Grade	179.2	8.15	39468
Tris Base, Molecular Biology Grade	121.1	8.30	648310
Tris Base, ULTROL® Grade	121.1	8.30	648311
Tris, HCl, Molecular Biology Grade	157.6	8.30	648317
Tris, HCl, ULTROL® Grade	157.6	8.30	648313
Trisodium Citrate, Dihydrate, Molecular Biology Grade	294.1	–	567446

1. pK_{a1} = 2.34; pK_{a2} = 9.6
2. pK_{a1} = 3.73; pK_{a2} = 7.96 (100 mM aqueous solution, 25 °C).
3. Phosphate buffers are normally prepared from a combination of the monobasic and dibasic salts, titrated against each other to the correct pH. Phosphoric acid has three pK_a values: pK_{a1}=2.12; pK_{a2}=7.21; pK_{a3}=12.32

* ULTROL® Grade - Buffers of uniform particle size and uniform solubility

Buffers, Buffer Capacity, and Range

Most simple buffers work effectively in the pH scale of $pK_a \pm 1.0$. Buffer capacity is a measure of the protection a buffer offers against changes in pH. Buffer capacity generally depends on the concentration of buffer solution. Buffers with higher concentrations offer higher buffering capacity. On the other hand, pH is dependent not on the absolute concentrations of buffer components, but on their ratio.

A buffer capacity of 1 is when one mole of acid or alkali is added to one liter of buffer and pH changes by one unit. The buffer capacity of a mixed weak acid-base buffer is much greater when the individual pK_a values are in close proximity with each other. It is important to note that the buffer capacity of a mixture of buffers is additive.

Using the above equation we know that when $pH = pK_a$ the concentrations of acetic acid and acetate ion are equal. Using a hypothetical buffer system of HA ($pK_a = 7.0$) and $[A^-]$, we can demonstrate how the hydrogen ion concentration, $[H^+]$, is relatively insensitive to external influence because of the buffering action.

For example:
If 100 mL of 10 mM (1×10^{-2} M) HCl are added to 1.0 liter of 1.0 M NaCl at pH 7.0, the hydrogen ion concentration, $[H^+]$, of the resulting 1.1 liter of solution can be calculated by using the following equation:

$$[H^+] \times Vol = [H^+]_o \times Vol_o$$

Where:

Vol_o = initial volume of HCl solution (in liters)
$[H^+]_o$ = initial hydrogen ion concentration (M)
Vol = final volume of HCl + NaCl solutions (in liters)
$[H^+]$ = final hydrogen ion concentration of HCl + NaCl solution (M)

Solving for $[H^+]$:

$$[H^+] \times 1.1 \text{ liter} = 1.0 \times 10^{-2} \times 0.1 = 1 \times 10^{-3}$$

$$[H^+] = 9.09 \times 10^{-4}$$

or

$$pH = 3.04$$

Thus, the addition of 1.0×10^{-3} mol of hydrogen ion resulted in a pH change of approximately 4 pH units (from 7.0 to 3.04). If a buffer is used instead of sodium chloride, a 1.0 M solution of HA at pK_a 7.0 will initially have:

$$[HA] = [A^-] = 0.5 \text{ M}$$

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

$$pH = 7.0 + \log \frac{0.5}{0.5} \quad \text{or} \quad pH = 7.0$$

When 100 mL of 1.0×10^{-2} M (10 mM) HCl is added to this system, 1.0×10^{-3} mol of A^- is converted to 1.0×10^{-3} mol of HA, with the following result:

$$pH = 7.0 + \log \frac{0.499/1.1}{0.501/1.1}$$

$$pH = 7.0 - 0.002 \quad \text{or} \quad pH = 6.998$$

Hence, it is clear that in the absence of a suitable buffer system there was a pH change of 4 pH units, whereas in a buffer system only a trivial change in pH was observed indicating that the buffer system had successfully resisted a change in pH. Generally, in the range from $[A^-]/[HA] = 0.1$ to $[A^-]/[HA] = 10.0$, effective buffering exists. However, beyond this range, the buffering capacity may be significantly reduced.

Buffering in Cells and Tissues

Buffers are important in biology – in fact, naturally occurring buffering systems in biological systems are crucial for hydrogen ion regulation. Amino acids present in proteins in cells and tissues contain functional groups that act as weak acid and bases. Nucleotides and several other low molecular weight metabolites that undergo ionization also contribute effectively to buffering in the cell. However, phosphate and bicarbonate buffer systems are most predominant in biological systems.

The phosphate buffer system has a pK_a of 6.86. Hence, it provides effective buffering in the pH range of 6.4 to 7.4. The bicarbonate buffer system plays an important role in buffering the blood system where in carbonic acid acts as a weak acid (proton donor) and bicarbonate acts as the conjugate base (proton acceptor) (Figure 2.1).

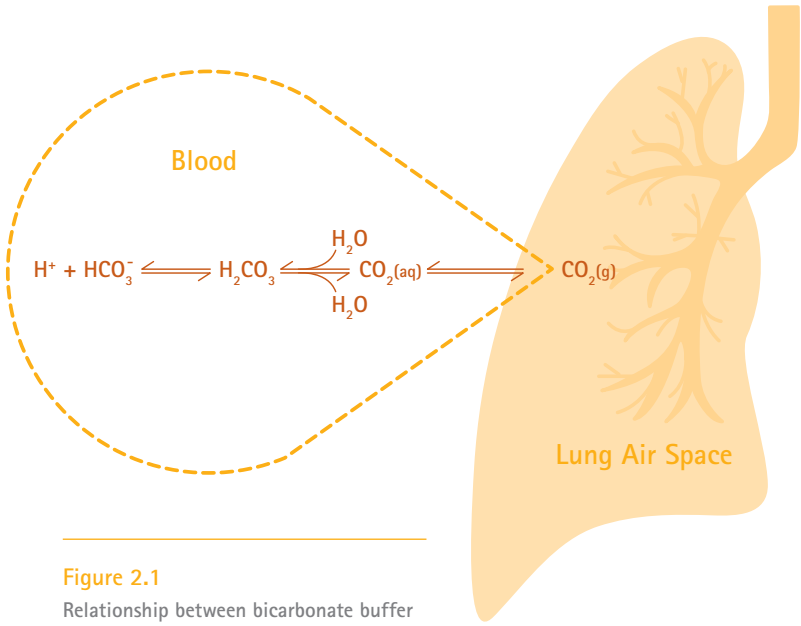


Figure 2.1
Relationship between bicarbonate buffer system and carbon dioxide

Effect of Temperature on pH

Generally when we consider the use of buffers we make the following two assumptions.

(a) The activity coefficients of the buffer ions is approximately equal to 1 over the useful range of buffer concentrations.

(b) The value of K_a is constant over the working range of temperature.

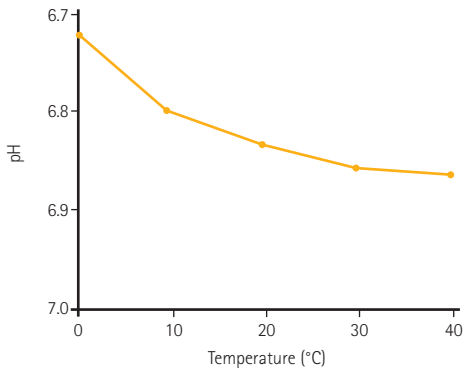


Figure 2.2
Effect of temperature on pH of Phosphate Buffer

However, in real practice one observes that pH changes slightly with change in temperature. This might be very critical in biological systems where a precise hydrogen ion concentration is required for reaction systems to operate with maximum efficiency. Figure 2.2 presents the effect of temperature on the pH of phosphate buffer. The difference might appear to be slight, but it has significant biological importance. Although the mathematical relationship of activity and temperature may be complicated, the actual change of pK_a with temperature (ΔpK_a/°C) is approximately linear. Table 2.2 presents the pK_a and ΔpK_a /°C for several selected zwitterionic buffers commonly used in biological experimentation.

Table 2.2 pK_a and ΔpK_a/°C of selected buffers

Buffer	M.W.	pK_a (20 °C)	pK_a (37 °C)	ΔpK_a/°C	Binding to Metal Ions
MES	195.2	6.15	5.97	–0.011	Negligible metal ion binding
ADA	212.2	6.60	6.43	-0.011	Cu^{2+}, Ca^{2+}, Mn^{2+}. Weaker binding with Mg^{2+}.
BIS-Tris Propane*	282.4	6.80	–	–0.016	–
PIPES	302.4	6.80	6.66	–0.009	Negligible metal ion binding
ACES	182.2	6.90	6.56	–0.020	Cu^{2+}. Does not bind Mg^{2+}, Ca^{2+}, or Mn^{2+}.
BES	213 .3	7.15	6.88	–0.016	Cu^{2+}. Does not bind Mg^{2+}, Ca^{2+}, or Mn^{2+}.
MOPS	209.3	7.20	6.98	–0.006	Negligible metal ion binding
TES	229.3	7.50	7.16	–0.020	Slightly to Cu^{2+}. Does not bind Mg^{2+}, Ca^{2+}, or Mn^{2+}.
HEPES	238.3	7.55	7.30	–0.014	None
HEPPS	252.3	8.00	7.80	–0.007	None
Tricine	179.2	8.15	7.79	–0.021	Cu^{2+}. Weaker binding with Ca^{2+}, Mg^{2+}, and Mn^{2+}.
Tris*	121 .1	8.30	7.82	–0.031	Negligible metal ion binding
Bicine	163.2	8.35	8.04	–0.018	Cu^{2+}. Weaker binding with Ca^{2+}, Mg^{2+}, and Mn^{2+}.
Glycylglycine	132.1	8.40	7.95	–0.028	Cu^{2+}. Weaker binding with Mn^{2+}.
CHES	207.3	9.50	9.36	–0.009	–
CAPS	221.32	10.40	10.08	–0.009	–

* Not a zwitterionic buffer

Selection and Use of Biological Buffers

Criteria for Suitable Biological Buffers

Biological buffers should meet the following general criteria:

- Their pK_a should be between 6.0 to 8.0.
- They should exhibit high water solubility and minimal solubility in organic solvents.
- They should not permeate cell membranes.
- They should not exhibit any toxicity towards cells.
- They should not interfere with any biological process.
- The salt effect should be minimal; however, salts can be added as required.
- Ionic composition of the medium and temperature should have minimal effect on buffering capacity.
- Buffers should be stable and resistant to enzymatic degradation.
- Buffers should not absorb either in the visible or in the UV region.

Most of the buffers used in cell cultures, isolation of cells, enzyme assays, and other biological applications must possess these distinctive characteristics. Good's zwitterionic buffers meet these criteria. They exhibit pK_a values at or near physiological pH. They exhibit low interference with biological processes due to the fact that their anionic and cationic sites are present as non-interacting carboxylate or sulfonate and cationic ammonium groups, respectively.

Choosing a Buffer

- Choose a buffer with a pK_a close to the desired working pH. If the pH is expected to decrease during the experiment, choose a buffer with a pK_a slightly lower than the working pH. Conversely, if the pH is expected to increase during the experiment, select a buffer with a pK_a slightly higher than the working pH.
- Adjust the pH at desired temperature. The pK_a of a buffer, and hence the pH, changes slightly with temperature. It is best to adjust the final pH at the desired temperature.
- Prepare buffers at working conditions. Always try to prepare your buffer solution at the temperature and concentration you plan to use during the experiment. If you prepare stock solutions, make dilutions just prior to use.

- Purity and cost. Compounds used should be stable and be available in high purity and at moderate cost.
- Spectral properties: Buffer materials should have no significant absorbance in the 240 to 700 nm range.
- Some weak acids (or bases) are unsuitable for use as buffers in certain cases. Citrate and phosphate buffers are not suitable for systems that are highly calcium-dependent. Citric acid and its salts are chelators of calcium and calcium phosphates are insoluble and will precipitate out. Use of these buffers may lower the calcium levels required for optimum reaction. Tris (hydroxymethyl) aminomethane is known to chelate calcium and other essential metals.
- Buffer materials and their salts can be used together for convenient buffer preparation. Many buffer materials are supplied both as a free acid (or base) and its corresponding salt. This is convenient when making a series of buffers with different pH. For example, solutions of 0.1 M HEPES and 0.1 M HEPES, sodium salt, can be mixed in an infinite number of ratios between 10:1 and 1:10 to provide 0.1 M HEPES buffer with pH values ranging from 6.55 to 8.55.
- Use stock solutions to prepare phosphate buffers. Mixing precalculated amounts of monobasic and dibasic sodium phosphates has long been established as the method of choice for preparing phosphate buffer. By mixing the appropriate amounts of monobasic and dibasic sodium phosphate solutions buffers in the desired pH range can be prepared.
- Adjust buffer materials to the working pH. Many buffers are supplied as crystalline acids or bases. The pH of these buffer materials in solution will not be near the pK_a, and the materials will not exhibit any buffering capacity until the pH is adjusted. In practice, a buffer material with a pK_a near the desired working pH is selected. If this buffer material is a free acid, pH is adjusted to desired working pH level by using a base such as sodium hydroxide, potassium hydroxide, or tetramethyl-ammonium hydroxide. Alternatively, pH for buffer materials obtained as free bases must be adjusted by adding a suitable acid.
- Use buffers without mineral cations when appropriate. Tetramethylammonium hydroxide fits this criterion. The basicity of this organic quaternary amine is equivalent to that of sodium or potassium hydroxide. Buffers prepared with this base can be supplemented at will with various inorganic cations during the evaluation of mineral ion effects on enzymes or other bioparticulate activities.
- Use a graph to calculate buffer composition. Figure 2.3 shows the theoretical plot of ΔpH versus $[A^-]/[HA]$ on two-cycle semilog paper. As most commonly used buffers exhibit only trivial deviations from theoretical value in the pH range, this plot can be of immense value in calculating the relative amounts of buffer components required for a particular pH. For example, suppose one needs 0.1 M MOPS buffer, pH 7.6 at 20 °C. At 20 °C, the pK_a for MOPS is 7.2. Thus, the working pH is about 0.4 pH units above the reported pK_a. According to the chart presented, this pH corresponds to a MOPS sodium/MOPS ratio of 2.5, and 0.1 M solutions of MOPS and MOPS sodium mixed in this ratio will give the required pH. If any significant deviations from theoretical values are observed, one should check the proper working conditions and specifications of their pH meter. The graph can also be used to calculate the amount of acid (or base) required to adjust a free base buffer material (or free acid buffer material) to the desired working pH.

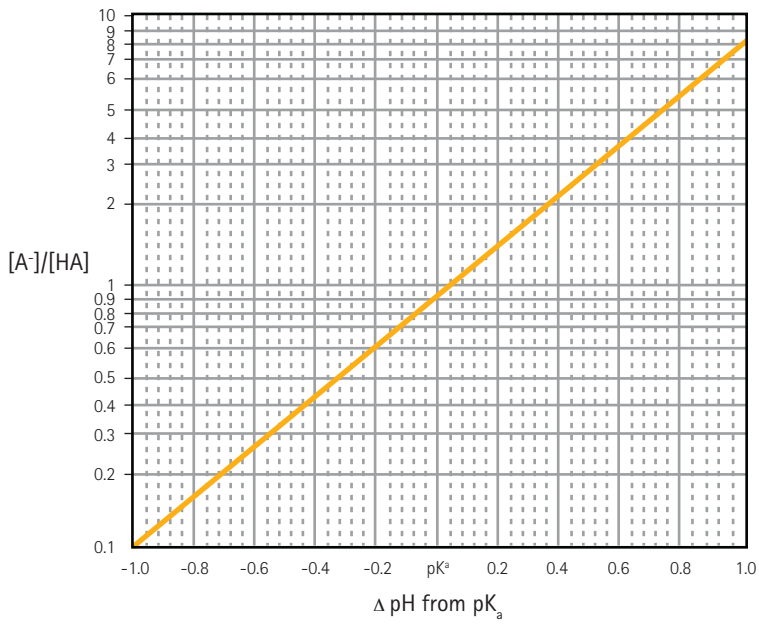


Figure 2.3
Theoretical plot of ΔpH versus $[A^-]/[HA]$ on two-cycle semilog graph

Buffer Interference with Reaction Systems

It is of utmost importance that researchers establish the criteria and determine the suitability of a particular buffer system. Some weak acids and bases may interfere with the reaction system, as shown in the following table:

Potentially unsuitable buffer	Reason for avoiding use
Citrate and phosphate buffers	Not recommended for systems that are highly calcium-dependent. Citric acid and its salts are powerful calcium chelators. Phosphates react with calcium producing insoluble calcium phosphate that precipitates out of the system.
Phosphate buffers	Phosphate ions in buffers can inhibit the activity of some enzymes, such as carboxypeptidase, fumarase, carboxylase, and phosphoglucomutase.
Tris(hydroxy-methyl) aminomethane, ACES, BES, and TES buffers	Can chelate copper and inhibit some enzymes.
Tris-based buffers	Not recommended when studying the metabolic effects of insulin.
HEPES and HEPPS	Not suitable when a protein assay is performed by using Folin reagent.
Buffers with primary amine groups, such as Tris	Interfere with the Bradford dye-binding method of protein assay.
Borate buffers	Not suitable for gel electrophoresis of protein, they can cause spreading of the zones if polyols are present in the medium.

Use of Water–Miscible Organic Solvents

Most pH measurements in biological systems are performed in the aqueous phase. However, sometimes mixed aqueous-water-miscible solvents, such as methanol or ethanol, are used for dissolving compounds of biological importance. These organic solvents have dissociation constants that are very low compared to that of pure water or of aqueous buffers (for example, the dissociation constant of methanol at 25 °C is 1.45×10^{-17}, compared to 1.0×10^{-14} for water). Small amounts of methanol or ethanol added to the aqueous medium will not affect the pH of the buffer. However, even small traces of water in methanol or DMSO can significantly change the pH of these organic solvents.

A Note on Isoelectric Point

The isoelectric point (pI) is the pH at which any given protein has an equal number of positive and negative charges. At a pH below this point, proteins carry a net positive charge, and above this point they have a net negative charge. This property has important biochemical implications in protein purification and electrophoresis. If the pH of the buffer is higher than the pI of the protein, it will migrate towards the positive terminal. On the other hand, if the pH of the buffer is lower than the pI of the protein, it will migrate towards the negative terminal. When the buffer pH is equal to the pI of a protein, it will not migrate at all.

A protein can exhibit different charges depending on the pH of the medium. At their pI, proteins exhibit the least electrostatic repulsion; hence they have the lowest solubility at this point and can easily precipitate. This property is useful in crystallization of proteins.
In general, positive and negative charges on the surface of proteins are balanced around neutral pH. The electrostatic attraction provides the compact shape and greater stability to the protein. However, at extremely low pH, the carboxyl groups are protonated and negative charges decrease. Here proteins gain more electrostatic repulsion and become denatured.

When acidic proteins are denatured in an acidic condition, they aggregate easily. On the other hand, when basic proteins are denatured in acidic conditions, they do not aggregate much because the proteins have greater number of positive charges in the acidic condition and the electrostatic repulsion is high. When the pH is brought back to neutral, non-precipitated basic proteins may return to their native structure. This is not generally true for precipitated acidic proteins, which often require treatment with strong denaturants, such as urea or guanidine hydrochloride.

Table 2.3 Isoelectric points of common proteins

Name	Organism/Tissue	Isoelectric Point	Molecular Weight
Acetylcholinesterase	Electric eel	4.5	230-260 kDa
α1-Acid glycoprotein	Human serum	1.8	~41 kDa
Acid protease	*Penicillium duponti*	3.9	~45 kDa
Aconitase	Porcine heart	8.5	~66 kDa
Adenosine deaminase	Human erythrocytes	4.7–5.1	~42 kDa
Adenylate cyclase	Mouse brain	5.9–6.1	~151 kDa
Adenylate kinase	Rat liver	7.5–8.0	~30.5 kDa
Adenylate kinase	Human erythrocytes	8.5–9.0	~22.5 kDa
Albumin	Human serum	4.6–5.3r	~67 kDa
Alcohol dehydrogenase	Horse liver	8.7–9.3	~149.5 kDa
Aldehyde dehydrogenase	Rat liver (cytosol)	8.5	~54 kDa
Aldolase	Rabbit muscle	8.2–8.6	~161 kDa
Alkaline phosphatase	Bovine intestine	4.4	~140 kDa
Alkaline phosphatase	Human liver	3.9	~156 kDa
β-Galactosidase	Rabbit brain	6.3	116 kDa
β-Glucuronidase	Rat liver microsomes	6.7	~75 – 78 kDa
cAMP-phosphodiesterase	Rat brain	6.1	~61-63 kDa
Amylase	Guinea Pig pancreas	8.4	~56 kDa
Amylase	Human saliva	6.2–6.5	~55.8 kDa
Arginase	Rat liver	9.4	~11.8 kDa
Arginase	Human liver	9.2	~10.7 kDa
ATPase (Na^+-K^+)	Dog heart	5.1	~99 kDa
Carbonic anhydrase	Porcine intestine	7.3	~30 kDa
Carboxypeptidase B	Human pancreas	6.9	~16 kDa
Carnitine acetyltransferase	Calf liver	6.0	~75 kDa
Catalase	Mouse liver (particulate)	6.7	~23.5 kDa
Cathepsin B	Human liver	5.1	~25 kDa
Cathepsin D	Bovine spleen	6.7	~42 kDa
Choline acetyltransferase	Human brain	7.8	~65 kDa
Collagenase	*Clostridium histolyticum*	5.5	~68-130 kDa
C-Reactive protein	Human serum	7.4	~115 kDa
DNA polymerase	Human lymphocytes	4.7	Polymerase I ~160 kDa
			Polymerase II ~30 kDa
DNase I	Bovine pancreas	4.7	~31 kDa
Dipeptidase	Porcine kidney	4.9	~300 kda
Enolase	Rat liver	5.9	~90 kDa
Epidermal growth factor	Mouse submaxillary glands	4.6	~6.1 kDa
Erythropoietin	Rabbit plasma	4.8–5.0	~10 – 30 kDa
Ferritin	Human liver	5.0–5.6	~ 450 kDa
α-Fetoprotein	Human serum	4.8	~69 kDa
Follicle stimulating hormone	Sheep pituitary	4.6	~33 kDa
Fructose 1,6-diphosphatase	Crab muscle	5.9	~135 kDa
γ-Glutamyl transpeptidase	Rat hepatoma	3.0	N\A
Galactokinase	Human placenta	5.8	~58 kDa
Glucose-6-phosphate dehydrogenase	Human erythrocytes	5.8–7.0	~240 kDa
Glutathione S-transferase	Rat liver	6.9, 8.1	~25.3 kDa

Name	Organism/Tissue	Isoelectric Point	Molecular Weight
D-Glyceraldehyde 3-phosphate dehydrogenase	Rabbit muscle	8.3	~35 kDa
L-Glycerol-3-phosphate dehydrogenase	Rabbit kidney	6.4	~50 kDa
Glycogen phosphorylase b	Human muscle	6.3	~94.5 kDa
Growth hormone	Horse pituitary	7.3	~20.2 kDa
Guanylate kinase	Human erythrocytes	5.1	~24 kDa
Hemoglobin	Rabbit erythrocyte	7.0	~65 kDa
Hemoglobin A	Human erythrocytes	7.0	~65 kDa
Hexokinase	Yeast	5.3	~50 - 100 kDa
Insulin	Bovine pancreas	5.7	~57.4 kDa
Lactate dehydrogenase	Rabbit muscle	8.5	~140 kDa
Leucine aminopeptidase	Porcine kidney	4.5	~255 kDa
Lipase	Human pancreas	4.7	~48 kDa
Malate dehydrogenase	Rabbit heart (cytosol)	5.1	~32 kDa
Malate dehydrogenase	Rabbit heart (mitochondria)	9.2	~36 - 48 kDa
Malic enzyme	Rabbit heart mitochondria	5.4	~180 kDa
Myoglobin	Horse muscle	7.3	~17 kDa
Nerve Growth Factor	Mouse salivary gland	9.3	~114 kDa
Ornithine decarboxylase	Rat liver	4.1	~54 kDa
Phosphoenolpyruvate carboxykinase	Mouse liver	6.1	~10 kDa
Phosphofructokinase	Porcine liver	5.0	~380 kDa
3-Phosphoglycerate kinase	Bovine liver	6.4	~49.6 kDa
Phospholipase A	Bee venom	10.5	~14.5 kDa
Phospholipase C	*C. perfringens*	5.3	~46 kDa
Phosphorylase kinase	Rabbit muscle	5.8	~31.8 kDa
Pepsin	Porcine stomach	2.2	~35 kDa
Plasmin	Human plasma	7.0–8.5	~47 kDa
Plasminogen	Human plasma	6.4–8.5	~86 kDa
Plasminogen proactivator	Human plasma	8.9	~17.5 kDa
Prolactin	Human pituitary	6.5	~22 kDa
Protein kinase A	Bovine brain	7.8	~49 kDa
Protein kinase A	Porcine brain	4.6	~35kDa
Prothrombin	Human plasma	4.6–4.7	~72 kDa
Pyruvate kinase	Rat liver	5.7	~60 kDa
Pyruvate kinase	Rat muscle	7.5	~58 kDa
Renin	Human kidney	5.3	~40 kDa
Ribonuclease	Bovine pancreas	9.3	~13.7 kDa
RNA polymerase II	Human	4.8	~250 kDa
Superoxide dismutase	*Pleurotus olearius*	7.0	~32.5 kDa
Thrombin	Human plasma	7.1	~37 kDa
Transferrin	Human plasma	5.9	~79.5 kDa
Trypsin inhibitor	Soybean	4.5	~20.1 kDa
Trypsinogen	Guinea Porcine pancreas	8.7	~24 kDa
Tubulin	Porcine brain	5.5	~55 kDa
Urease	Jack bean	4.9	~480 kDa

Preparation of Some Common Buffers for Use in Biological Systems

The information provided below is intended only as a general guideline. We strongly recommend the use of a sensitive pH meter with appropriate temperature setting for final pH adjustment. Addition of other chemicals, after adjusting the pH, may change the final pH value to some extent. The buffer concentrations in the tables below are used only as examples. You may select higher or lower concentrations depending upon your experimental needs.

1. Hydrochloric Acid-Potassium Chloride Buffer (HCl-KCl); pH Range 1.0 to 2.2

(a) 0.1 M Potassium chloride: 7.45 g/L (M.W.: 74.5)

(b) 0.1 M Hydrochloric acid

Mix 50 mL of potassium chloride and indicated volume of hydrochloric acid. Mix and adjust the final volume to 200 mL with deionized water. Adjust the final pH using a sensitive pH meter.

mL of HCl	97	64.5	41 .5	26.3	16.6	10.6	6.7
pH	1.0	1.2	1.4	1.6	1.8	2.0	2.2

2. Glycine-HCl Buffer; pH range 2.2 to 3.6

(a) 0.1 M Glycine: 7.5 g/L (M.W.: 75.0)

(b) 0.1 M Hydrochloric acid

Mix 50 mL of glycine and indicated volume of hydrochloric acid. Mix and adjust the final volume to 100 mL with deionized water. Adjust the final pH using a sensitive pH meter.

mL of HCl	44.0	32.4	24.2	16.8	11.4	8.2	6.4	5.0
pH	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6

3. Citrate Buffer; pH range 3.0 to 6.2

(a) 0.1 M Citric acid: 19.21 g/L (M.W.: 192.1)

(b) 0.1 M Sodium citrate dihydrate: 29.4 g/L (M.W.: 294.0)

Mix citric acid and sodium citrate solutions in the proportions indicated and adjust the final volume to 100 mL with deionized water. Adjust the final pH using a sensitive pH meter. The use of pentahydrate salt of sodium citrate is not recommended.

mL of Citric acid	46.5	40.0	35.0	31.5	25.5	20.5	16.0	11.8	7.2
mL of Sodium citrate	3.5	10.0	15.0	18.5	24.5	29.5	34.0	38.2	42.8
pH	3.0	3.4	3.8	4.2	4.6	5.0	5.4	5.8	6.2

4. Acetate Buffer; pH range 3.6 to 5.6

(a) 0.1 M Acetic acid (5.8 mL made to 1000 mL)

(b) 0.1 M Sodium acetate; 8.2 g/L (anhydrous; M.W. 82.0) or 13.6 g/L (trihydrate; M.W. 136.0)

Mix acetic acid and sodium acetate solutions in the proportions indicated and adjust the final volume to 100 mL with deionized water. Adjust the final pH using a sensitive pH meter.

mL of Acetic acid	46.3	41.0	30.5	20.0	14.8	10.5	4.8
mL of Sodium acetate	3.7	9.0	19.5	30.0	35.2	39.5	45.2
pH	3.6	4.0	4.4	4.8	5.0	5.2	5.6

5. Citrate-Phosphate Buffer; pH range 2.6 to 7.0

(a) 0.1 M Citric acid; 19.21 g/L (M.W. 192.1)

(b) 0.2 M Sodium phosphate, Dibasic; 35.6 g/L (dihydrate; M.W. 178.0) or 53.6 g/L (heptahydrate; M.W. 268.0)

Mix citric acid and sodium phosphate solutions in the proportions indicated and adjust the final volume to 100 mL with deionized water. Adjust the final pH using a sensitive pH meter.

mL of Citric acid	44.6	39.8	35.9	32.3	29.4	26.7	24.3	22.2	19.7	16.9	13.6	6.5
mL of Sodium phosphate, Dibasic	5.4	10.2	14.1	17.7	20.6	23.3	25.7	27.8	30.3	33.1	36.4	43.6
pH	2.6	3.0	3.4	3.8	4.2	4.6	5.0	5.4	5.8	6.2	6.6	7.0

6. Phosphate Buffer; pH range 5.8 to 8.0

(a) 0.1 M Sodium phosphate, Monobasic; 13.8 g/L (monohydrate, M.W. 138.0)

(b) 0.1 M Sodium phosphate, Dibasic; 26.8 g/L (heptahydrate, M.W. 268.0)

Mix sodium phosphate monobasic and dibasic solutions in the proportions indicated and adjust the final volume to 200 mL with deionized water. Adjust the final pH using a sensitive pH meter.

mL of Sodium phosphate, Monobasic	92.0	81.5	73.5	62.5	51.0	39.0	28.0	19.0	13.0	8.5	5.3
mL of Sodium phosphate, Dibasic	8.0	18.5	26.5	37.5	49.0	61.0	72.0	81.0	87.0	91.5	94.7
pH	5.8	6.2	6.4	6.6	6.8	7.0	7.2	7.4	7.6	7.8	8.0

7. Tris-HCl Buffer, pH range 7.2 to 9.0

(a) 0.1 M Tris(hydroxymethyl)aminomethane; 12.1 g/L (M.W.: 121.0)

(b) 0.1 M Hydrochloric acid

Mix 50 mL of Tris(hydroxymethyl)aminomethane and indicated volume of hydrochloric acid and adjust the final volume to 200 mL with deionized water. Adjust the final pH using a sensitive pH meter.

mL of HCl	44.2	41.4	38.4	32.5	21.9	12.2	5.0
pH	7.2	7.4	7.6	7.8	8.2	8.6	9.0

8. Glycine-Sodium Hydroxide, pH 8.6 to 10.6

(a) 0.1 M Glycine; 7.5 g/L (M.W.: 75.0)

(b) 0.1 M Sodium hydroxide; 4.0 g/L (M.W.: 40.0)

Mix 50 mL of glycine and indicated volume of sodium hydroxide solutions and adjust the final volume to 200 mL with deionized water. Adjust the final pH using a sensitive pH meter.

mL of Sodium hydroxide	4.0	8.8	16.8	27.2	32.0	38.6	45.5
pH	8.6	9.0	9.4	9.8	10.0	10.4	10.6

9. Carbonate-Bicarbonate Buffer, pH range 9.2 to 10.6

(a) 0.1 M Sodium carbonate (anhydrous), 10.6 g/L (M.W.: 106.0)

(b) 0.1 M Sodium bicarbonate, 8.4 g/L (M.W.: 84.0)

Mix sodium carbonate and sodium bicarbonate solutions in the proportions indicated and adjust the final volume to 200 mL with deionized water. Adjust the final pH using a sensitive pH meter.

mL of Sodium carbonate	4.0	9.5	16.0	22.0	27.5	33.0	38.5	42.5
mL of Sodium bicarbonate	46.0	40.5	34.0	28.0	22.5	17.0	11.5	7.5
pH	9.2	9.4	9.6	9.8	10.0	10.2	10.4	10.6

10. Krebs-Henseleit bicarbonate buffer, pH 7.4

119 mM NaCl

4.7 mM KCl

2.5 mM $CaCl_2$

1.2 mM $MgSO_4$

1.2 mM KH_2PO_4

25 mM $NaHCO_3$

pH 7.4 (at 37 °C) when equilibrated with 95% O_2 and 5% CO_2. Adjust the pH before use.

11. Hank's Biocarbonate Buffer, pH 7.4

137 mM NaCl

5.4 mM KCl

0.25 mM Na_2HPO_4

0.44 mM KH_2PO_4

1.3 mM $CaCl_2$

1.0 mM $MgSO_4$

4.2 mM $NaHCO_3$

pH 7.4 (at 37 °C) when equilibrated with 95% O_2 and 5% CO_2. Adjust the pH before use.

12. Phosphate-Buffered Saline (PBS), pH 7.4

150 mM NaCl

10 mM Potassium Phosphate buffer
(1 liter PBS can be prepared by dissolving 8.7 g NaCl, 1.82 g $K_2HPO_4 \bullet 3H_2O$, and 0.23 g KH_2PO_4 in 1 liter of distilled water.)

Adjust the pH before use.

A variation of PBS can also be prepared as follows:

137 mM NaCl

2.7 mM KCl

10 mM Na_2HPO_4

1.76 mM KH_2PO_4

13. Tris Buffered Saline (TBS), pH 7.4

10 mM Tris

150 mM NaCl

(1 liter of TBS can be prepared by dissolving 1.21 g of Tris base and 8.7 g of NaCl in 1 liter of distilled water. Adjust the pH before use. Note: Tris has a pK_a of 8.3. Hence, the buffering capacity at pH 7.4 is minimal compared to phosphate buffer (pK_a = 7.21).

14. TBST (Tris Buffered Saline and TWEEN®-20)

10 mM Tris-HCl, pH 8.0

150 mM NaCl

0.1% TWEEN®-20

15. Stripping Buffer for Western Blotting Applications

62.5 mM Tris buffer, pH 6.7 to 6.8

2% Sodium dodecyl sulfate (SDS)

100 mM β-Mercaptoethanol

16. Cell Lysis Buffer

20 mM Tris-HCl (pH 7.5)

150 mM NaCl

1 mM Sodium EDTA

1 mM EGTA

1% TRITON® X-100

2.5 mM Sodium pyrophosphate

1 mM β-Glycerophosphate

1 mM Sodium orthovanadate

1 µg/mL Leupeptin

Reference Tables for Buffer Formulation

Table 2.4 Approximate pH and bicarbonate concentration in extracellular fluids

Fluid	pH	mEq HCO_3^-/liter
Plasma	7.35–7.45	28
Cerebrospinal fluid	7.4	25
Saliva	6.4–7.4	10–20
Gastric secretions	1.0–2.0	0
Tears	7.0–7.4	5–25
Aqueous humor	7.4	28
Pancreatic juice	7.0–8.0	80
Sweat	4.5–7.5	0–10

Table 2.5 Ionic composition of body fluids

	[mEq/L]	
Ion	Plasma	Intracellular Fluid
Sodium	142	10
Potassium	4	160
Calcium	5	2
Magnesium	2	26
Chloride	101	3
Bicarbonate	27	10
Phosphate	2	100
Sulfate	1	20

Table 2.6 Ionization constants K and pK_a for selected acids and bases in water

Acids and Bases	Ionization Constant (K)	pK_a
Acetic Acid	1.75×10^{-5}	4.76
Citric Acid	7.4×10^{-4}	3.13
	1.7×10^{-5}	4.77
	4.0×10^{-7}	6.40
Formic Acid	1.76×10^{-4}	3.75
Glycine	4.5×10^{-3}	2.35
	1.7×10^{-10}	9.77
Imidazole	1.01×10^{-7}	6.95
Phosphoric Acid	7.5×10^{-3}	2.12
	6.2×10^{-8}	7.21
	4.8×10^{-13}	12.32
Pyruvic Acid	3.23×10^{-3}	2.49
Tris(hydroxymethyl)aminomethane	8.32×10^{-9}	8.08

Table 2.7 Physical properties of some commonly used acids

Acid	Molecular Weight	Specific Gravity	%Weight/Weight	Approx.Normality	mL required to make 1 liter of 1 N solution
Acetic Acid	60.05	1.06	99.50	17.6	57
Hydrochloric Acid	36.46	1.19	37	12.1	83
Nitric Acid	63.02	1.42	70	15.7	64
Perchloric Acid (72%)	100.46	1.68	72	11.9	84
Phosphoric Acid	98.00	1.70	85	44.1	23
Sulfuric Acid	98.08	1.84	96	36.0	28

Some Useful Tips

pH Measurements

- A pH meter may require several minutes to warm up. When a pH meter is routinely used in the laboratory, it is better to leave it "ON" with the function switch at "standby."
- Set the temperature control knob to the temperature of your buffer solution. Always warm or cool your buffer to the desired temperature before checking final pH.
- Before you begin make sure the electrode is well rinsed with deionized water and wiped off with a clean absorbent paper.
- Always rinse and wipe the electrode when switching from one solution to another.
- Calibrate your pH meter by using at least two standard buffer solutions.
- Do not allow the electrode to touch the sides or bottom of your container.
- When using a magnetic bar to stir the solution, make sure the electrode tip is high enough to prevent any damage.
- Do not stir the solution while taking the reading.
- Inspect your electrode periodically. The liquid level should be maintained as per the specification provided with the instrument.
- Glass electrodes should not be left immersed in solution any longer than necessary. This is important especially when using a solution containing proteins. After several pH measurements of solutions containing proteins, rinse the electrode in a mild alkali solution and then wash several times with deionized water.
- Water used for preparation of buffers should be of the highest possible purity. Water obtained by a method combining deionization and distillation is highly recommended.
- To avoid any contamination, do not store water for longer than necessary. Store water in tightly sealed containers to minimize the amount of dissolved gases.
- One may sterile-filter the buffer solution to prevent any bacterial or fungal growth. This is important when large quantities of buffers are prepared and stored over a long period of time.

Some Useful Tips

Calculation of Concentrations and Spectrophotometric Measurements

A = abc

Where A = absorbance

a = proportionality constant defined as absorptivity

b = light path in cm

c = concentration of the absorbing compound

When b is 1 cm and c is moles/liter, the symbol a is substituted by the symbol ε(epsilon).

ε is a constant for a given compound at a given wavelength under prescribed conditions of solvent, temperature, pH and is called as molar absorptivity. ε is used to characterize compounds and establish their purity.

Example:

Bilirubin dissolved in chloroform at 25 °C should have a molar absorptivity ε of 60,700.

Molecular weight of bilirubin is 584.

Hence 5 mg/Liter (0.005 g/L) read in 1 cm cuvette should have an absorbance of:

A = (60,700)(1)(0.005/584) = 0.52 {A = abc}

Conversely, a solution of this concentration showing absorbance of 0.49 should have a purity of 94% (0.49/0.52).

In most biochemical and toxicological work, it is customary to list constants based on concentrations in g/dL rather than mol/liter. This is also common when molecular weight of the substance is not precisely known.

Here for b = 1 cm; and c = 1 g/dL (1%), A can be written as:

$$A^{1\%}_{cm}$$

This constant is known as absorption coefficient.

The direct proportionality between absorbance and concentration must be established experimentally for a given instrument under specified conditions.

Frequently there is a linear relationship up to a certain concentration. Within these limitations, a calibration constant (K) may be derived as follows:

A = abc

Therefore,

c = A/ab = A x 1/ab

The absorptivity (a) and light path (b) remain constant in a given method of analysis. Hence, 1/ab can be replaced by a constant (K).

Then,

c = A x K; where K = c/A

The value of the constant K is obtained by measuring the absorbance (A) of a standard of known concentration (c).

EMD Millipore Calbiochem® Buffers

Advantages

- Are available in various grades and package sizes for greater flexibility
- Offer highly purified substances, to ensure reproducible results from the lab bench to production
- Are trusted, as demonstrated through thousands of citations
- Are manufactured in a highly controlled environment, ensuring the utmost purity and quality

We are continuing to expand our line of OmniPur® and ULTROL® grade buffer materials, which are of superior quality and are manufactured to meet our stringent specifications. ULTROL® grade buffers, whenever possible, are screened for uniform particle size, giving uniform solubility characteristics. OmniPur® grade reagents are DNase, RNase, and protease tested to ensure the integrity of your starting material and to improve downstream results.

Available from: **www.emdmillipore.com**

Phosphate-Buffered Saline (PBS) Tablets (Catalogue No. 524650)

Developed for use in immunoassays, PBS Tablets are very convenient for preparing PBS buffer solutions. PBS has been used for many applications due to its isotonic properties and nontoxic characteristics. PBS is most commonly used as a diluent and as a wash buffer for cells and immunoassays. PBS can also be used to take a reference spectrum in spectroscopic analysis of proteins. Dissolving one tablet in 1 liter of deionized H_2O yields 140 mM NaCl, 10 mM phosphate buffer, and 3 mM KCl, pH 7.4 at 25 °C. Note: 10 tablets/pack.

HEPES, Free Acid, ULTROL® Grade (Catalogue No. 391338)

A zwitterionic N-substituted aminosulfonic acid buffer, useful in the pH 6.8-8.2 range. Has a pK_a of 7.48 at 25 °C. Absorbance (1.0 M, H_2O, 260 nm): ≤0.05. It is one of the twelve Good's buffers. HEPES is commonly used in cell culture applications. Its ability to maintain physiological pH despite changes in carbon dioxide concentration is superior when compared to other bicarbonate buffers also used in cell culture.

Tris Base, ULTROL® Grade (Catalogue No. 648311)

Tris is an abbreviation of the organic compound known as tris(hydroxymethyl)aminomethane. Tris (THAM) is extensively used in biochemistry and molecular biology as a component of buffer solutions, such as in TAE and TBE buffer, especially for solutions of nucleic acids. Useful in the pH range of 7.0-9.0. Has a pK_a of 8.1 at 25 °C. Absorbance (1.0 M, H_2O, 260 nm): <0.05.

IHC Select® Citrate Buffer pH 6.0, 10x (Catalogue No. 21545)

For use as a pretreatment reagent in immunohistochemical staining procedures after dilution. Use prepared 1X Citrate Buffer to retrieve epitopes in formalin-fixed, paraffin-embedded tissue sections mounted on glass microscope slides. This procedure is performed after deparaffinization and prior to immunohistochemistry (IHC).

Key Products

Description	Size	Catalogue No.
10X PBS, Liquid, OmniPur®	4 L	6505-4L
Tris, OmniPur®	5 kg	9230-5KG
MOPS, Free Acid, ULTROL® grade	1 kg	475898
PBS-TWEEN® Tablets	10 tablets	524650
HEPES, Free Acid, ULTROL® grade	1 kg	391338

For more key buffers and buffer components from EMD Millipore, see page 67.

Related Products

Stericup® and Steritop® Filter Units for Buffer Sterilization

Eliminating contaminants from your buffers, cell culture media and additives is crucial to preserving the accuracy and integrity of biological research. Featuring high-performance membranes, Stericup® and Steritop® filters are the most trusted devices for sterile filtration of volumes ranging from 150 mL to 1,000 mL. Stericup® filters combine a filter unit with a receiver flask and cap for processing and storage and provide superior recovery. Steritop® bottle top filters can be used with bottles having a 33 mm or 45 mm opening.

Key Benefits

- Both filter units available with fast flow Millipore Express® PLUS and low-protein-binding Durapore® membranes.
- No loss of media in the filtration process
- 0.01 µm pore size available for mycoplasma removal

Description	Catalogue No.
Stericup®-GP 0.22 PES Filter, 500 mL	SCGPU05RE
Stericup®-GP 0.22 PES Filter, 1000 mL	SCGPU11RE
Stericup®-GV 0.22 Durapore Filter, 500 mL	SCGVU05RE
Stericup®-GV 0.22 Durapore Filter, 1000 mL	SCGVU11RE

3 Detergents

This chapter provides information on the use of detergents in biological systems. The background information here, though not comprehensive, will hopefully serve the needs of the first-time users of detergents as well as the needs of experienced investigators.

Also included is a section on a unique series of compounds known as Non-Detergent Sulfobetaines (NDSBs). As evident from the name, these compounds are not detergents and they do not form micelles. Structurally, NDSBs have hydrophilic groups similar to those found in zwitterionic detergents; however, they possess a much shorter hydrophobic chain. They have been reported to improve the yield of membrane proteins when used in conjunction with the traditional detergents and prevent aggregation during renaturation of chemically or thermally denatured proteins.

Importance of Detergents in Biochemistry

Hydrophobic Interactions

Hydrophobic interactions play a major role in defining the native tertiary structure of proteins. Proteins consist of polar and non-polar amino acids. In water-soluble proteins, hydrophobic domains rich in non-polar amino acids are folded in together and thus are shielded from the aqueous environment. In membrane proteins, some hydrophobic regions that otherwise would be exposed to the aqueous environment are surrounded by lipids.

What are Detergents?

Detergents are amphipathic molecules that contain both polar and hydrophobic groups. Because of their amphipathic nature, detergents are able to solubilize hydrophobic compounds in water and promote water solubility of hydrophobic proteins, such as membrane proteins, while maintaining their folded form. These molecules contain a polar group (head) at the end of a long hydrophobic carbon chain (tail). In contrast to purely polar or non-polar molecules, amphipathic molecules exhibit unique properties in water. Their polar group forms hydrogen bonds with water molecules, while the hydrocarbon chains aggregate due to hydrophobic interactions. These properties allow detergents to be soluble in water.

In aqueous solutions, detergents form organized spherical structures called micelles (Figure 3.1), each of which contain several detergent molecules. Micelles are formed at or above a threshold concentration called the critical micelle concentration (CMC). The CMC can be determined by measuring the ability of a detergent to solubilize a hydrophobic dye by light scattering, or by determining surface tension. Detergents are also known as surfactants because they decrease the surface tension of water.

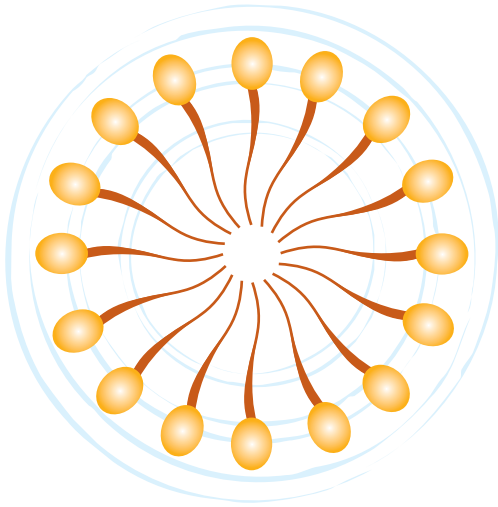


Figure 3.1
A detergent-micelle in water

Biological Membranes

Biological membranes are complex assemblies of lipids and proteins. They serve as physical barriers between and within cells, and are sites of many signaling events. Most membrane lipids contain two hydrophobic groups connected to a polar head. This molecular architecture allows lipids to form lipid bilayers, in which the hydrophobic chains face each other while the polar head groups face outward to the aqueous milieu. Proteins and lipids, like cholesterol, are embedded in this bilayer. This bilayer model for membranes is known as the fluid mosaic model (Figure 3.2); "fluid" because components can move laterally, and "mosaic" because of the diversity of the bilayer's components. Integral membrane proteins are held in the membrane by hydrophobic interactions between the hydrocarbon chains of the lipids and the hydrophobic domains of the proteins. These integral membrane proteins are insoluble in water but are soluble in detergent solutions.

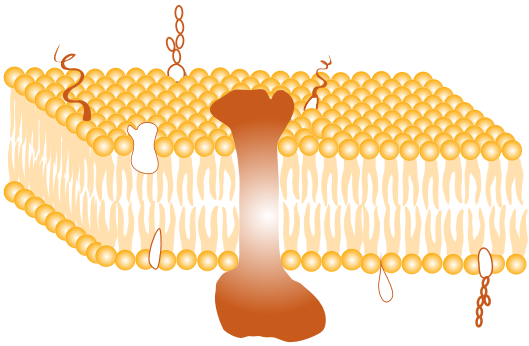


Figure 3.2
Fluid-mosaic model of a biological membrane

To understand the structure and function of membrane proteins, it is necessary to carefully isolate and thoroughly purify these proteins in their native form. About one third of all membrane-associated proteins are integral membrane proteins, and their solubilization and purification is even more challenging because most of them are present in very low concentrations. Although membrane protein solubilization can be accomplished by using amphiphilic detergents, preservation of their biological and functional activities can be challenging because many membrane proteins are susceptible to denaturation during the isolation process.

How Do Detergents Solubilize Membrane Proteins?

Detergents solubilize membrane proteins by mimicking the lipid-bilayer environment. Micelles formed by detergents are analogous to the bilayers of the biological membranes. Proteins incorporate into these micelles via hydrophobic interactions. Hydrophobic regions of membrane proteins, normally embedded in the membrane lipid bilayer, are surrounded by a layer of detergent molecules and the hydrophilic portions are exposed to the aqueous medium. This keeps the membrane proteins in solution. Complete removal of detergent could result in protein aggregation due to the clustering of hydrophobic regions, causing precipitation of membrane proteins.

Although phospholipids can be used as detergents in simulating the bilayer environment, they form large structures, called vesicles, which are not easily amenable to isolation and characterization of membrane proteins. Lysophospholipids form micelles that are similar in size to those formed by many detergents. However, they are too expensive to be of general use in everyday protein biochemistry. Hence, the use of synthetic detergents is highly preferred for the isolation of membrane proteins.

Dissolution of membranes by detergents can be divided into different stages (Figure 3.3). At low concentrations, detergents bind to the membrane by partitioning into the lipid bilayer. At higher concentrations, when the bilayers are saturated with detergents, the membranes disintegrate to form mixed micelles with the detergent molecules. In the detergent-protein mixed micelles, hydrophobic regions of the membrane proteins are surrounded by the hydrophobic chains of micelles. In the final stages, solubilization of the membranes leads to the formation of mixed micelles consisting of lipids and detergents and detergent micelles containing proteins (usually one protein molecule per micelle). For example, solubilization of a membrane containing rhodopsin by digitonin leads to complexes containing one rhodopsin molecule per micelle consisting of 180 digitonin molecules. Other combinations of micelles containing lipids and detergents and lipid-protein-detergent molecules are possible at intermediate concentrations of detergent. Micelles containing protein-detergent molecules can be separated from other micelles based on their charge, size, or density.

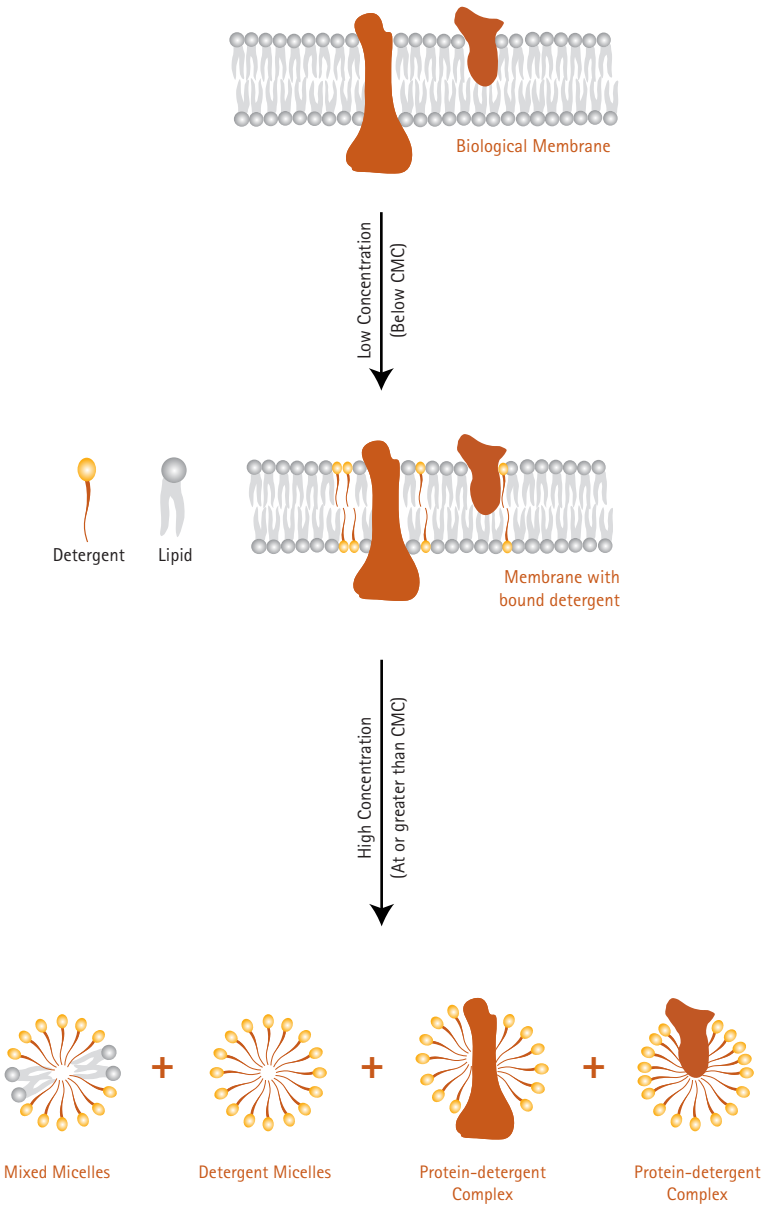


Figure 3.3
Stages in the dissolution of a biological membrane with detergents.

Importance of Detergent–Lipid–Protein Ratios for Successful Membrane Protein Solubilization

- At low detergent concentration, monomers merely bind to the membrane, and there is minimal membrane perturbation.
- At higher detergent concentration, membrane lysis occurs and lipid-protein-detergent mixed micelles are generated.
- Much higher detergent concentration generates heterogeneous complexes of detergent, lipid, and protein. Progressive delipidation of lipid-protein detergent mixed micelles occurs, which forces lipids to distribute among the increasing concentration of detergent micelles. This gives rise to lipid/detergent and protein/detergent mixed micelles.
- With increased detergent concentration, a steady-state point is reached. Above this point solubilization does not increase any further and activity of the protein begins to decline.

Solubilization of the membrane is often accompanied by selective or differential solubilization of membrane lipids (due to asymmetric extraction of membrane lipids by detergents). This means that certain lipids could be enriched. For example, cholesterol, sphingomyelin, and glycolipids are enriched when red blood cells are extracted with TRITON® X-100 detergent.

Classification of Detergents

A large number of detergents with various combinations of hydrophobic and hydrophilic groups are now commercially available. Based on the nature of the hydrophilic head group, they can be broadly classified as ionic, nonionic, and zwitterionic detergents.

Ionic Detergents

Ionic detergents contain a head group with a net charge. They can be either negatively (anionic) or positively charged (cationic). For example, sodium dodecyl sulfate (SDS), which contains the negatively charged sulfate group, is an anionic detergent while cetyltrimethyl-ammonium bromide (CTAB), which carries the positively charged trimethylammonium group, is a cationic detergent. Furthermore, ionic detergents either contain a hydrocarbon (alkyl) straight chain as in SDS and CTAB, or a more complicated rigid steroidal structure as in sodium deoxycholate (see bile acid salts). There is repulsion between similarly charged polar groups of detergent molecules in a micelle. Therefore, the size of the micelle is determined by the combined effect of hydrophobic attraction of the side chains and the repulsive forces of the ionic groups. Consequently, neutralizing the charge on the head group with increasing concentrations of a counter ion leads to a larger micellar size. Micellar size also increases with the increase in alkyl chain length.

Bile Acid Salts

Bile acid salts are anionic detergents containing a rigid steroidal hydrophobic group (e.g. sodium salts of cholic acid and deoxycholic acid). In addition to the anionic carboxyl group at the end of the short alkyl chain they also carry hydroxyl groups on the steroid structure. Thus, there is no well-defined polar head group. Instead, the bean-shaped molecule has a polar and an apolar face. Bile acid salts form small aggregates. They can be conjugated to taurine or glycine at the end of the carboxyl group. Unlike spherical micelles formed by alkyl ionic detergents, the micelles formed by bile acid salts are kidney-shaped due to their rigid structure. Similar to the behavior of ionic detergents, the micellar size of bile acid salts is influenced by the concentration of the counter ion. Due to the low pK_a (5–6) of the unconjugated bile salt and low solubility of bile acids, their utility is limited to the alkaline pH range. On the other hand, the pK_a of conjugated bile acid salts is much lower; hence, they can be used over a broad pH range. Dihydroxy bile acid salts and deoxycholate are more effective than trihydroxy bile acid salts in membrane solubilization and in dissociation of protein-protein interactions. Trihydroxy bile acid salts are milder and are better suited for removal by dialysis.

Nonionic Detergents

Nonionic detergents contain uncharged, hydrophilic head groups that consist of either polyoxyethylene moieties as in BRIJ® and TRITON® Detergents or glycosidic groups as in octyl glucoside and dodecyl maltoside. In general, nonionic detergents are better suited for breaking lipid-lipid and lipid-protein interactions than protein-protein interactions. Hence, they are considered nondenaturing and are widely used in the isolation of biologically active membrane proteins. Unlike ionic detergents, salts have minimal effect on the micellar size of the nonionic detergents.

Detergents with polyoxyethylene head groups may contain alkylpolyethylene ethers with the general formula $C_nH_{2n+1}(OCH_2CH_2)_xOH$, or a phenyl ring between the alkyl chain and the ether group. TRITON® X-100 and NP-40 Detergents belong to the latter class (see Table 3.1 on page 30). Polyoxyethylene chains form random coils and are consequently farther removed from the hydrophobic core of the micelles. Detergents with shorter polyoxyethylene chains form aggregates and viscous solutions in water at room temperature, whereas those with longer chains do not aggregate. Detergents containing aromatic rings absorb light in the ultraviolet region and may interfere with spectrophotometric monitoring of proteins at 280 nm. Hydrogenated versions of these detergents are also available, in which the aromatic rings are reduced and these detergents exhibit relatively low absorption at 280 nm. Infrared-based detection, such as in EMD Millipore's Direct Detect™ quantitation system, can be used to accurately quantitate proteins in the presence of detergents and other lipids, which do not interfere with the portion of the IR spectrum used to quantitate proteins.

Alkyl glycosides have become more popular as nonionic detergents in the isolation of membrane proteins for several reasons. First, they exhibit homogeneous composition. Second, several variations of alkyl glycosides containing different combinations of the hydrocarbon chain (cyclic or straight chain) and the polar sugar group can be easily synthesized in pure forms. Third, subtle differences in the physicochemical properties of alkyl glycosides bearing various alkyl chains, attached to either to a glucose, maltose, or a sucrose head group, can be exploited for selective solubilization of membrane proteins.

Zwitterionic Detergents

Zwitterionic detergents are unique in that they offer the combined properties of ionic and nonionic detergents. Like nonionic detergents, the zwitterionic detergents, including CHAPS and the ZWITTERGENT® Detergent 3-X series, do not possess a net charge — they lack conductivity and electrophoretic mobility, and do not bind to ion-exchange resins. However, like ionic detergents, they are efficient at breaking protein-protein interactions. Zwitterionic detergents such as CHAPS are less denaturing than the ZWITTERGENT® Detergent 3-X series, possibly owing to their rigid steroid ring structure.

Table 3.1 Types of detergents

Types of Detergents	Examples	Main Features
Ionic Detergents	• Anionic: Sodium dodecyl sulfate (SDS) • Cationic: Cetylmethyl ammonium bromide (CTAB)	• Contain head group with a net charge • Micelle size is determined by the combined effect of hydrophobic attraction of the side chain and the repulsive force of the ionic head group • Neutralizing the charge on the head group with increasing counter ions can increase micellar size • Useful for dissociating protein-protein interactions • CMC is reduced by increasing the ionic strength of the medium, but is relatively independent of temperature
Nonionic Detergents	• TRITON®-X-100 Detergent • *n*-octyl-β-D-glucopyranoside	• Uncharged hydrophilic head group • Better suited for breaking lipid-lipid and lipid-protein interactions • Considered to be nondenaturing • Salts have minimal effect on micellar size • Solubilize membrane proteins in a gentler manner, allowing the solubilized proteins to retain native subunit structure, enzymatic activity and/or non-enzymatic function • CMC is relatively unaffected by increasing ionic strength, but increases substantially with rising temperature
Zwitterionic Detergents	• CHAPS • ZWITTERGENT® detergents	• Offer combined properties of ionic and nonionic detergents • Lack conductivity and electrophoretic mobility • Do not bind to ion exchange resins • Suited for breaking protein-protein interactions

Non-Detergent Sulfobetaines

The unique non-detergent sulfobetaines (NDSBs) are zwitterionic compounds that are very useful for protein chemistry. Like Zwittergent® Detergents, NDSBs carry the sulfobetaine hydrophilic head group. However, in contrast to Zwittergent® Detergents, the hydrophobic group in NDSBs is too short for micellar formation even at concentrations as high as 1 M. Hence, they do not behave like detergents. NDSBs were first employed in native isoelectrofocusing gels to neutralize electrostatic interactions without increasing the conductivity. NDSBs are zwitterionic over a wide pH range and can easily be removed by dialysis. They do not absorb significantly at 280 nm.

NDSBs have proven useful in several applications, including isolation of membrane proteins and purification of nuclear and halophilic proteins. Presumably, the contribution from the short hydrophobic groups combined with the charge neutralization ability of the sulfobetaine group result in higher yields of membrane proteins. NDSBs have also been used in renaturation and refolding of chemically and thermally denatured proteins. NDSBs prevent protein aggregation and improve the yield of active proteins when added to the buffer during *in vitro* protein denaturation. The hydrophobic group, although short, may interact with the hydrophobic regions of the protein to prevent aggregation during renaturation. NDSBs have been used in renaturation of fusion proteins from inclusion bodies.

NDSBs do not interfere with enzymatic assays involving chromogenic substrates bearing nitrophenyl groups and they do not inhibit the activities of β-galactosidase or alkaline phospatase. In addition, NDSB-195, NDSB-211, and NDSB-221 do not absorb at 280 nm, making them compatible with protein purification procedures in which the protein concentrations are monitored by measuring absorbance at 280 nm.

Additional References for NDSBs:

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Vuillard, L., et al. 1995 . Biochem. J. 305, 337.
Vuillard, L., et al. 1998 . Eur. J. Biochem. 256, 128 .

Product	M.W.	Catalogue No.
NDSB-195	195.3	480001
NDSB-201	201.2	480005
NDSB-211	211.3	480013
NDSB-221	221.3	480014
NDSB-256	257.4	480010
NDSB-256-4T	257.4	480011
NDSB-Set (includes NDSB-195, 201, 256, 211)	NA	480012

Critical Micelle Concentration (CMC)

The CMC can be defined as the lowest concentration above which monomers cluster to form micelles. Alternatively, it is the maximum attainable chemical potential (concentration) of the monomer. In reality, micellization occurs over a narrow concentration range rather than at a particular concentration.

Factors that increase CMC	Factors that decrease CMC
Short alkyl chain	Longer alkyl chain
Double bonds	
Branch points in chemical structure (such as those occurring in bile acid salts)	
For nonionic detergents, increasing temperature	For ionic detergents, increasing counterion concentration

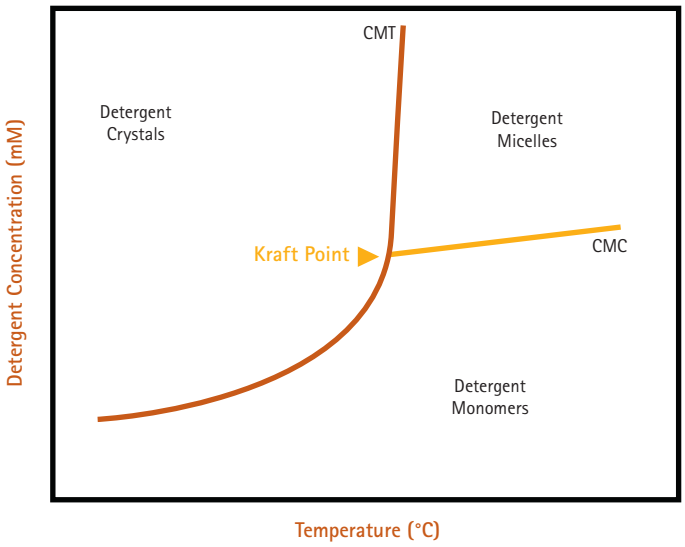


Figure 3.4
Temperature-composition phase diagram for detergent solutions.

From a practical point of view, use a detergent with a high CMC when dialysis is used for the removal of the detergent.

Given the CMC, the concentration of the detergent and the aggregation number (see page 33), it is possible to calculate the concentration of micelles in moles per liter using the following formula:

$$[\text{micelles}] = (Cs - CMC) \div N$$

where Cs is the bulk molar concentration of detergent and N is the mean aggregation number. For example, a solution containing 35 mM of CHAPSO (MW. = 630.9) in PBS buffer will have [(35 – 8) ÷ 11] or 2.45 mM of micelles.

Kraft Point

The temperature at which all the three phases—crystalline, micellar, and monomeric — exist in equilibrium is called the Kraft Point (Figure 3.4). At this temperature, the detergent solution turns clear and the concentration of the detergent reaches its CMC value. At very low temperatures, detergents remain mainly in an insoluble crystalline state and are in equilibrium with small amounts of dissolved monomer. As the temperature increases, more and more of the monomeric detergent goes into solution until the concentration of the detergent reaches the CMC. At this point it exists predominantly in the micellar form. The temperature at which the monomer reaches the CMC concentration is called the critical micellar temperature (CMT). For most detergents, the Kraft point is identical to the CMT.

Cloud Point

At a particular temperature above the CMT, nonionic detergents become cloudy and undergo phase separation to yield a detergent-rich layer and an aqueous layer. This temperature is called the cloud point. Phase separation presumably occurs due to a decrease in hydration of the head group. For example, the cloud point of TRITON® X-100 Detergent is 64 °C whereas that for TRITON® X-114 Detergent is around 22 °C. Hence, TRITON® X-114 Detergent solutions are kept cold. This property can be used to a particular advantage. Membranes can be first solubilized at 0 °C and the solution can be warmed to about 30 °C to effect the phase separation. This allows partition of integral membrane proteins into the detergent-rich phase, which can be separated later by centrifugation.

Aggregation Number

This is the number of monomeric detergent molecules contained in a single micelle. It can be obtained by dividing the micellar molecular weight by the monomeric molecular weight. The molecular weight of micelles can be obtained from various techniques including gel filtration, light scattering, sedimentation equilibrium, and small-angle X-ray scattering. The micelles formed by bile acid salts tend to have low aggregation numbers while those formed by TRITON® have high aggregation numbers. Like micellar size, the aggregation number is also influenced by the ionic strength.

$$\frac{\text{micellular molecular weight}}{\text{monomeric molecular weight}} = \text{aggregation number}$$

Hydrophile–Lipophile Balance (HLB)

The HLB, hydrophilic-lipophilic balance, is a measure of the relative hydrophobicity of the detergent. There is a good correlation between the HLB value of a detergent and its ability to solubilize membrane proteins. The most hydrophobic detergents have a HLB number approaching zero, while the least hydrophobic detergents have values reaching 20. Detergents with a HLB value in the range 12 to 20 are preferred for non-denaturing solubilization. For example, solubility of D-alanine carboxypeptidase correlates well with the HLB number, while no correlation exists with CMC or surface tension. Detergents with HLB number between 12-14 were most effective (Umbreit and Strominger, 1973*). Detergents in the higher end of the range are preferred for solubilization of extrinsic proteins.

It is important to note that the HLB is additive. For example, when two detergents with HLB values of A and B are used the following equation applies.

$$HLB\ (A+B) = (Ax + By)/x+y$$

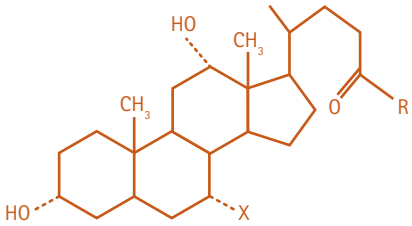
where x and y are the percentages of each detergent. Provided there are no other factors influencing enzyme activity, using the above formula, two detergents can be selected to attain the desired HLB value.

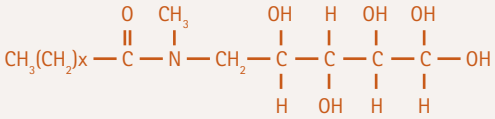
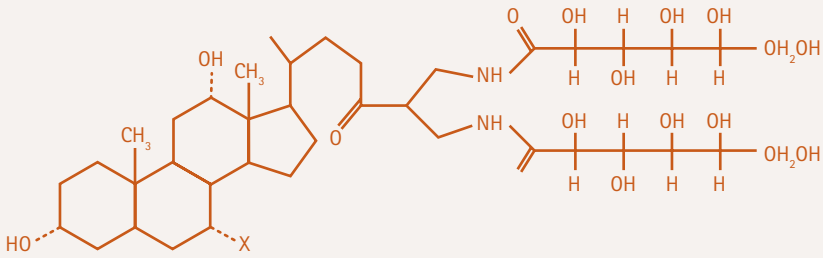
Summary of Factors Affecting Detergent Performance

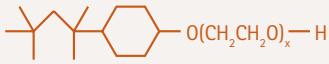
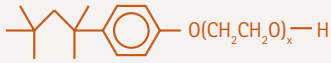
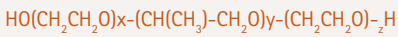
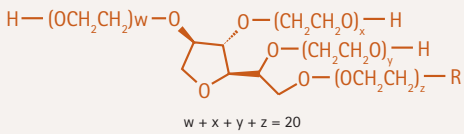
- Detergent concentration
- Ionic strength
- Length of the alkyl chain
- pH
- Presence of organic additives
- Purity
- Temperature

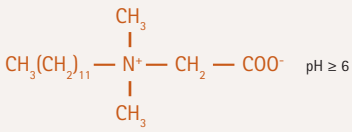
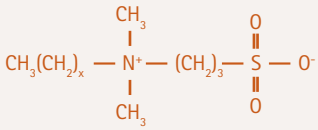
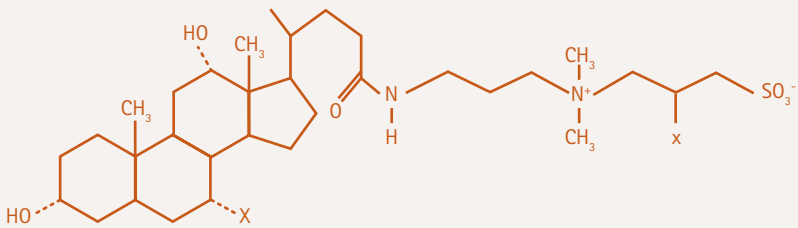
*Umbreit, JN and Strominger, JL. 1973 . Proc. Natl. Acad. Sci. USA; vol. 70:2997.

Table 3.2 Detergent classes, structures, and examples

Detergent Class	General Structure	Examples
Alkyl glycosides	R-O-$(CH_2)_x$-CH_3	R = glucose x = 8, *n*-nonyl-β-D-glucopyranoside x = 7, *n*-octyl-β-D-glucopyranoside x = 6, *n*-heptyl-β-D-glucopyranoside x = 5, *n*-hexyl-β-D-glucopyranoside
	R -S-$(CH_2)_x$-CH_3	R = maltose x = 11, dodecyl-β-D-maltoside x = 9, decyl-β-D-maltoside
		R = glucose, x = 7, octyl-β-D-thioglucopyranoside
Bile acids		x = H, R = O-Na^+, sodium deoxycholate x = H, R = $NHCH_2CH_2SO_3$-Na^+, sodium taurodeoxycholate x = H, R = $NHCH_2CO_2$-Na^+, sodium glycodeoxycholate x = OH, R = O-Na^+, sodium cholate x = OH, R = $NHCH_2$ CH_2SO_3-Na^+, sodium taurocholate x = OH, R = $NHCH_2CO_2$-Na^+, sodium glycocholate

Detergent Class	General Structure	Examples
Glucamides		x = 8, MEGA-10 x = 7, MEGA-9 x = 6, MEGA-8
		x = H, Deoxy Big CHAP x = OH, Big CHAP

Detergent Class	General Structure	Examples
Poly-oxyethylenes, monodisperse and polydisperse		x = 9–10, reduced TRITON® X-100 x = 7–8, reduced TRITON® X-114
		x = 9–10, TRITON® X-100, NP-40 x = 7–8, TRITON® X-114
		y = 12, x = 8, GENAPOL® X-080 y = 12, x = 10, GENAPOL® X-100 y = 11 , x = 8, $C_{12}E_8$ y = 11 , x = 9, $C_{12}E_9$, THESIT™, LUBROL® PX y = 11 , x = 10, GENAPOL® C-100 y = 11 , x = 23, BRIJ® 35
		x = 98, y = 67, z = 98, PLURONIC® F-127®
	 w + x + y + z = 20	R= $C_{11}H_{23}CO_2$- (laurate), TWEEN® 20 R=$C_{17}H_{33}CO_2$- (oleate), TWEEN® 80

Detergent Class	General Structure	Examples
Zwitterionic Detergents		EMPIGEN® BB (*n*-dodecyl-N,N-dimethylglycine)
		x = 7, ZWITTERGENT® 3-08 x = 9, ZWITTERGENT® 3-10 x = 11, ZWITTERGENT® 3-12 x = 13, ZWITTERGENT® 3-14 x = 15, ZWITTERGENT® 3-16
		x = H, CHAPS x = OH, CHAPSO

Removal of Unbound Detergents

Excess detergent is normally used to solubilize membrane proteins. This is to ensure complete dissolution of the membrane and to provide a large number of micelles such that only one protein molecule is present per micelle. However, for further physicochemical and biochemical characterization of membrane proteins, it is often necessary to remove the unbound detergent. Several detergent removal methods take advantage of the general properties of detergents: hydrophobicity, CMC, aggregation number, and the charge. The following is a brief description of five commonly used methods.

Hydrophobic Adsorption

This method exploits the ability of detergents to bind to hydrophobic resins. For example, certain hydrophobic resins that are insoluble can be used in batchwise applications to remove excess detergent. Generally, a solution containing a detergent is mixed with a specific amount of the resin and the mixture is allowed to stand at 4 °C or room temperature. The resin with the bound detergent can be removed by centrifugation or filtration. This technique is effective for removal of most detergents. If the adsorption of the protein to the resin is of concern, the resin can be included in a dialysis buffer and the protein dialyzed. However, dialysis against resin may take a long time.

Table 3.3 Percent detergent removal after one spin with Amicon® Ultra-4 centrifugal devices

		After filtration with:	
Detergent	Original concentration	10 kDa MWCO	30 kDa MWCO
SDS	0.1%	95%	98%
	1.0%	38%	48%
	5.0%	94%	95%
TWEEN®-20	0.1%	30%	42%
	1.0%	28%	35%
	5.0%	82%	77%
TRITON® X-100	0.1%	3%	47%
	1.0%	2%	20%
	5.0%	2%	20%
CHAPS	0.1%	90%	96%
	1.0%	66%	93%
	5.0%	38%	73%

Gel Chromatography

Gel chromatography takes advantage of the difference in size between protein-detergent, detergent-lipid, and homogeneous detergent micelles. In most situations, protein-detergent micelles elute in the void volume. The elution buffer should contain a detergent below its CMC value to prevent protein aggregation and precipitation. Separation by gel chromatography is based on size. Hence, parameters that influence micellar size (ionic strength, pH, and temperature) should be kept constant from experiment to experiment to obtain reproducible results.

Centrifugal Ultrafiltration

Amicon® Ultra centrifugal filters are efficient laboratory tools for removing small molecules from solutions of proteins or nucleic acids. Micelle formation results in aggregation of the detergent and leads to gross changes in molecular structure. This affects the amount of the detergent that can be removed from a solution by centrifugal devices with specific molecular weight cut off (MWCO) membranes.

For example, the monomer of TRITON® X-100 has a molecular weight of 500–650 daltons. TRITON® X-100 should pass readily through the 10,000 MWCO membrane in an Amicon® Ultra device. However, at concentrations above 0.01% (0.2 mM), TRITON® X-100 forms micelles composed of approximately 140 monomeric units. During ultrafiltration, the micelles behave like 70,000–90,000 dalton globular proteins.

As a result, more than 90% of TRITON® X-100 is retained by the ultrafiltration membrane. Therefore, above the CMC of TRITON® X-100, an Amicon® Ultra-4 filter with MWCO of 100,000 would be required to remove the detergent effectively.

Dialysis

When detergent solutions are diluted below the CMC, the micelles are dispersed into monomers. The size of the monomers is usually an order of magnitude smaller than that of the micelles and thus can be easily removed by dialysis. If a large dilution is not practical, micelles can be dispersed by other techniques such as the addition of bile acid salts. For detergents with a high CMC, dialysis is usually the preferred choice.

Ion-exchange Chromatography

This method exploits the differences in charge between protein-detergent micelles and protein-free detergent micelles. When nonionic or zwitterionic detergents are used, conditions can be chosen so that the protein-containing micelles are adsorbed on the ion-exchange resin and the protein-free micelles pass through. Adsorbed protein is washed with detergent-free buffer and is eluted by changing either the ionic strength or the pH. Alternatively, the protein can be eluted with an ionic detergent thus replacing the nonionic detergent.

Guidelines for Choosing a Detergent

A membrane protein is considered solubilized if it is present in the supernatant after one hour centrifugation of a lysate or a homogenate at 100,000 x g. In most cases, the biological activity of the protein should be preserved in the supernatant after detergent solubilization. Hence, the appropriate detergent should yield the maximum amount of biologically active protein in the supernatant. Given the large number of detergents available today, choosing an appropriate detergent can be a difficult process. Some of the points outlined below can be helpful in selecting a suitable detergent.

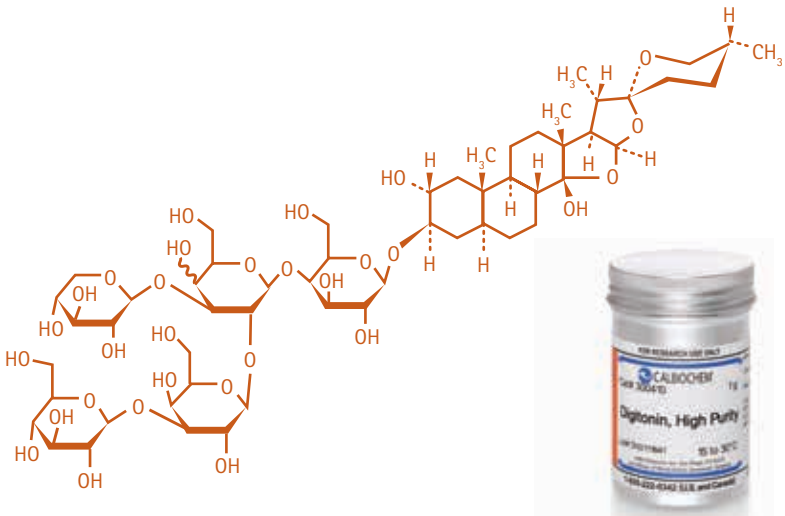
- First, survey the literature. Try a detergent that has been used previously for the isolation and characterization of a protein with similar biochemical or enzymological properties.
- Consider the solubility of the detergent at the working temperature. For example, ZWITTERGENT® 3-14 Detergent is insoluble in water at 4 °C while TRITON® X-114 Detergent undergoes a phase separation at room temperature.
- Consider the method of detergent removal. If dialysis is to be employed, a detergent with a high CMC is clearly preferred. Alternatively, if ion exchange chromatography is utilized, a nonionic detergent or a ZWITTERGENT® Detergent is the detergent of choice.
- Preservation of biological or enzymological activity may require experimenting with several detergents. Not only the type, but also the quantity of the detergent used will affect the protein activity. For some proteins biological activity is preserved over a very narrow range of detergent concentration. Below this range the protein is not solubilized and above a particular concentration, the protein is inactivated.
- Consider downstream applications. Since TRITON® X-100 Detergent contains aromatic rings that absorb at 260-280 nm, this detergent should be avoided if the protocols require UV monitoring of protein concentration. Similarly, ionic detergents should be avoided if the proteins are to be separated by isoelectric focusing. For gel filtration of proteins, detergents with smaller aggregation numbers should be considered.
- Consider detergent purity. Detergents of utmost purity should be used since some detergents such as TRITON® X-100 Detergent are generally known to contain peroxides as contaminants. The Calbiochem® PROTEIN GRADE® or ULTROL® grade detergents that have been purified to minimize these oxidizing contaminants are recommended.
- A variety of Molecular Biology Grade detergents, including EMD Millipore's OmniPur® line of biochemicals, are available for any research where contaminants such as DNase, RNase, and proteases are problematic.
- A nontoxic detergent should be preferred over a toxic one. For example, digitonin, a cardiac glycoside, should be handled with special care.
- For as yet unknown reasons, specific detergents often work better for particular isolation procedures. For example, *n*-Dodecyl-β-D-maltoside (EMD Millipore Catalogue No. 324355) has been found to be the detergent of choice for the isolation of cytochrome c oxidase. Hence, some "trial and error" may be required for determining optimal conditions for isolation of a membrane protein in its biologically active form.
- Sometimes it is difficult to find an optimally suited detergent for both solubilization and analysis of a given protein. In such cases, it is often possible to solubilize proteins with one detergent before replacing it with another that exhibits least interference with analysis.
- In some cases, it has been observed that the inclusion of non-detergent sulfobetaines (NDSBs) with detergents in the isolation buffer dramatically improves yields of solubilized membrane proteins.

EMD Millipore Calbiochem® Brand Detergents

Advantages

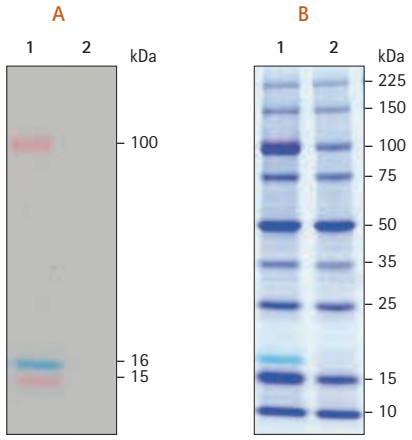
- Available in various grades and package sizes for greater flexibility
- Offer highly purified substances, to ensure reproducible results from the lab bench to production
- Are trusted, as demonstrated through thousands of citations
- Manufactured in a highly controlled environment, ensuring the utmost purity and quality

Available from: **www.emdmillipore.com**



Digitonin, High Purity (Catalogue No. 300410)

Digitonin permeabilizes plasma membranes of eukaryotic cells by complexing with membrane cholesterol and other unconjugated beta-hydroxysterols. It is a nonionic detergent commonly used to solubilize membrane-bound proteins. Digitonin is an ideal tool for membrane protein research, and has proven to be especially useful in the study of neurotransmitter receptors (dopamine receptors, β-adrenaline receptors, GABA receptors, acetylcholine receptors). This detergent, a cardiac glycoside, should be handled with special care as it belongs to family of toxic detergents.



(A) Unstained gel and Western transfer 4–20% SDS-PAGE

(B) Coomassie blue 4–20% SDS-PAGE

Lane 1: Trail Mix™ Protein Markers

Lane 2: Perfect Protein™ Markers only

OmniPur® Sodium Dodecyl Sulfate (SDS), also known as sodium laurilsulfate (Catalogue No. 7910-500GM)

SDS, commonly found in consumer products such as toothpaste, shampoos, or bubblebath foam, is widely used in biochemistry. It can be used to lyse cells during DNA extraction and to unfold proteins for SDS-PAGE. SDS denatures proteins by disrupting non-covalent bonds that maintain native conformation.

DetergentOUT™ Detergent Removal Kits (Catalogue Nos. 2110, 2112, 2114, 2116)

DetergentOUT™ Kits are a simple high performance method for removing ionic detergents such as SDS from protein solutions without significant dilution. Simply load the protein solution on the DetergentOUT™ GBS10 spin column and collect detergent-free protein solution. Micro columns (Catalogue No. 2114) are suitable for removing detergent from a maximum of 0.2 mL sample volume. Medium columns (Catalogue No. 2116) are suitable for removing detergent from a maximum of 0.5 mL sample volume.

Key Products

Description	Size	Catalogue No.
Digitonin, High Purity	5 g	300410
CHAPS, OmniPur®	100 g	3055-100GM
Sodium Dodecyl Sulfate, OmniPur®	500 g	7910-500GM
ZWITTERGENT™ 3-14 Detergent	100 g	693017
TWEEN® 20, OmniPur®	1 L	9480-1L
DetergentOUT™ GBS10	1 kit	2110
Detergent-OUT™ GBS10 Med Kit	1 kit	2112
Detergent-OUT™ Micro Kit	1 kit	2114
Detergent-OUT™ Med Kit	1 kit	2116

For more key detergents from EMD Millipore, see page 70.

TECHNOLOGY HIGHLIGHT

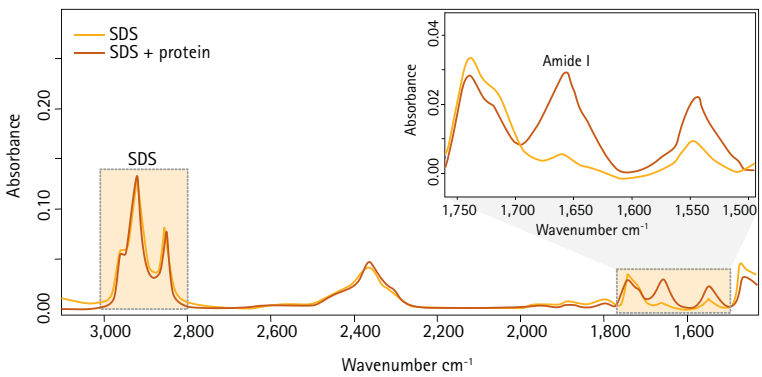
Accurate protein quantitation in presence of detergents using the Direct Detect™ spectrometer

The Direct Detect™ system, an infrared (IR)-based spectrometry system, represents an innovation in biomolecular quantitation. It employs a novel hydrophilic polytetrafluoroethylene (PTFE) membrane that is designed to be transparent in most of the infrared spectral region and enables application of biomolecule solutions directly onto the membrane. By measuring amide bonds in protein chains, the system accurately determines an intrinsic component of every protein without relying on amino acid composition, dye-binding properties or redox potential.

Unlike traditional colorimetric assays used for protein quantiation, the Direct Detect™ system can be used to accurately measure protein concentration in the presence of detergents, such as SDS, NP40 and Tween® 20, commonly used for protein purification and stabilization.

IR-based protein quantitation using the Direct Detect™ system involves measuring the intensity of the Amide I signal in the protein's IR spectrum, and subtracting the signal contributed by buffer alone in that region. A sample spectrum (below) shows that the IR spectrum of SDS does not have a strong signal in the Amide I region that would significantly interfere with protein quantitation. As a result, IR-based quantitation retains accuracy and reproducibility in the presence of SDS.

Similarly, the IR spectra of other detergents, such as Tween® 20, Triton® X-100 and NP40, and reducing agents such as DTT do not interfere with Amide I quantitation (spectra not shown).



The characteristic peaks of the SDS IR spectrum are distinct from the Amide I region of the protein spectrum.

4 Dyes and Stains

Cell visualization and microscopy are fundamental tools for investigating cellular behavior and structure, including changes in structural features, as well as cellular and sub-cellular dynamics under normal and pathological conditions. As the technology for microscopy has evolved, the ability to interrogate discrete protein and organelle dynamics has increased and the need for both general and specific visualization tools has grown. Dyes and stains provide rich information on cellular and subcellular processes, especially when used in conjunction with fluorescently-conjugated primary and secondary antibodies.

The main purpose of dyes and stains in biological applications is to make microscopic objects more clearly visible than they would be if they were not stained. Stains are developed to specifically stain certain molecules or subcellular components, enabling discrimination from other components of the cell, as well as for quantitative comparison between samples.

Fluorescent dyes, or fluorochromes, absorb energy when exposed to incident light of a particular wavelength, causing excitation of electrons to higher energy levels. Subsequently, some of the excess energy is emitted as light when electrons return to ground level. By selective excitation of fluorochromes, one can detect signals with high specificity and sensitivity using fluorescence microscopy or flow cytometry.

Types of Dyes

Histological Stains

Most tissue sections are transparent and lack contrast if viewed using light or electron microscopy. Therefore, histological stains are used to distinguish morphology of cells and tissues. Common light microscopy stains include hematoxylin, a basic molecule that stains nuclei, and eosin, an acidic dye that stains the cytoplasm pink. Other stains include safranin, crystal violet, malachite green, and Giemsa stains.

Table 4.1 Common histological stains and their uses

Histological Stain	Use
Cresyl Blue	Stains reticulocytes in hematology samples
Giemsa Stain Solution, Original Azure Blend	Staining G-bands of chromosomes in genetic studies
Schiff Reagent	Carbohydrate staining
Crystal Violet	Gram staining: stains gram positive bacteria purple
Safranin	Stains Gram-negative bacteria red
Alizarin Red S	Gross staining of skeletons in small vertebrates, differentiating bone from cartilage in mammalian embryos.
Aniline Blue	Staining cytoplasm, cellulose, and determining hydrolysis of fats.
Brilliant Green	Chemical indicator, pH range 0.0-2.6. Color change, yellow to green.
Methyl Green	Differentiation of DNA and RNA
Methylene Blue	General histological and bacteriological staining

Chromogenic Stains

Chromogenic stains are frequently used for enzyme-mediated histological staining, in conjunction with specific antibodies conjugated to reporter enzymes. Examples of such chromogenic stains include AEC (3-amino-9-ethylcarbazole), DAB (3,3'-Diaminobenzidine), and TMB (3,3',5,5´-Tetramethylbenzidine). The solubility of a histological stain in alcohol determines whether an alcohol-based or an aqueous counterstain should be used to complete the histological analysis. Table 4.2 lists common chromogenic stains, their alcohol solubility, and common applications.

Table 4.2 Common chromogenic stains, their alcohol solubility, and common applications

Chromogenic stain	Abbreviation	Final Color	Soluble in Alcohol	Comments
Diaminobenzidine	DAB	Brown	No	3,3'-Diaminobenzidine produces a brown end product which is highly insoluble in alcohol and other organic solvents. Oxidation of DAB also causes polymerization. DAB has the ability to react with osmium tetroxide, and thus is very useful in electromicroscopy as well as traditional immunohistochemistry sections.
Diaminobenzidine with nickel enhancement	DAB/Nickel	Gray/Black	No	Produces a more intense stain, which is resistant to alcohol and provides better contrast, up to 40 times more sensitive than DAB without enhancement.
3-Amino-9-ethylcarbazole	AEC	Red/Brown	Yes	AEC produces a red/brown reaction product and is widely used for immunohistochemical staining. Slide specimens processed with AEC must not be immersed in alcohol or alcoholic solutions (e.g., Harris' hematoxylin). Instead, an aqueous counterstain and mounting medium should be used. AEC is also susceptible to further oxidation when exposed to light and thus it will fade overtime. Dark storage and brief light viewing are recommended.
4-Chloro-1-naphthol	4-CN	Blue/Gray	Yes	4-Chloro-1-naphthol (CN) precipitates as a blue/gray end product. Because CN is soluble in alcohol and other organic solvents, the slides must not be dehydrated, exposed to alcoholic counterstains, or coverslipped with mounting media containing organic solvents. Unlike DAB, CN tends to diffuse from the site of precipitation, thus it is not usually recommended for Immunohistochemistry but can be used for Western blotting.
Naphthol AS B1 phosphate/ Fast Red TR	NABP/FR	Red	Yes	Napthol AS- acts as the substrate for alkaline phosphate/Fast Red TR phosphatase, and the Fast Red chromogen precipitates at the enzymatic sites producing a vibrant red/pink color. Precipitate is soluble in alcohol, thus aqueous counterstain and mounting medium should be used.
Naphthol AS MX phosphate/ Fast Red TR	NAMP/FR	Red	Yes	Napthol AS- acts as the substrate for alkaline phosphatase, and the Fast Red chromogen precipitates at the enzymatic sites producing a vibrant red/pink color. Precipitate is soluble in alcohol thus aqueous counterstain and mounting medium should be used.
Naphthol AS B1 phosphate/ New Fuschin	NABP/NF	Red/Violet	Yes	Napthol AS- acts as the substrate for alkaline phosphatase, and the new Fuchsin chromogen precipitates at the enzymatic sites producing a vibrant red/violet color. Precipitate is soluable in alcohol, thus aqueous counterstain and mounting medium should be used.
Bromochloroindolyl phosphate Nitro Blue Tetrazolium	BCIP/NBT	Purple	No	5-Bromo-4-chloro-3-indolyl phosphate (BCIP)/Nitro Blue Tetrazolium (NBT) substrate is a commonly used substrate chromogen. BCIP acts as the substrate for alkaline phosphatase, and the NBT enhances the purplish-brown color of the precipitate. BCIP/NBT is compatible with organic solvents so it can be used with alcohol based counterstains including Nuclear Fast Red or Methylene-Green.

Fluorescent DNA-Binding Dyes

Because many cellular processes involve changes in chromatin organization and changes in the DNA-rich nucleus, visualizing DNA using fluorescent dyes is an effective method of tracking cell viability, cell cycle, cell proliferation, and apoptosis. Visualization of the nucleus is also important for determining relative localization of detected biomolecules and subcellular structures. Finally, DNA-binding dyes are frequently used during molecular cloning and DNA amplification to determine the size and purity of DNA fragments or to visualize fragments for subsequent processing.

Dyes (and other molecules, for that matter) bind to DNA in different structural modes (Figure 4.1). Intercalating dyes, usually made up of planar chemical structures and aromatic rings, bind via aromatic stacking between base pairs of DNA. Examples of intercalating dyes are ethidium bromide (EtBr) and propidium iodide (PI). Minor groove-binding dyes are another large group of DNA binders, and include 4′,6-Diamidino-2-phenylindole (DAPI), a common nuclear stain for immunocytochemistry) and Hoechst dyes. Functional groups on dyes can confer some sequence specificity; for example, hydrogen bonding functional groups on chromomycin A3 make this minor-groove-binding dye bind preferentially to GC-rich regions.

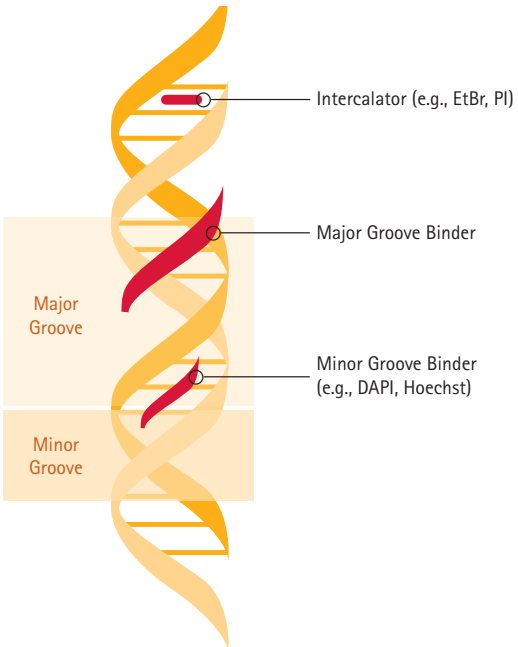


Figure 4.1
Structural modes of DNA binding.

Fluorochromes for Covalently Labeling Biomolecules

Amine-reactive dyes

Because conjugation to amine groups generates very stable labels, amine-reactive dye probes (such as dansyl chloride, FITC, and Texas Red are most commonly used to generate fluorescent protein or nucleic acid conjugates for applications in which relatively harsh conditions may be applied to the labeled biomolecule, such as immunochemistry, fluorescence *in situ* hybridization (FISH), cell tracing, and receptor labeling for cell signaling studies. These experiments often involve stringent washing, permeabilization, fixation, and mounting, potentially involving organic solvents or reactive chemicals. Amine-conjugated dyes have the best chance of remaining bound and retaining fluorescent properties throughout these processing steps, enabling informative detection and analysis.

Amine-reactive dyes are typically conjugated to protein lysine residues and/or to the free amine at the N-terminus. When labeling a protein with an amine-reactive dye, the pH can affect the efficiency and specificity of the labeling reaction. Lysines are efficiently modified in pH of 8.5 to 9.5. On the other hand, one can selectively modify a protein's N-terminus (pK_a of ~7) by carrying out the labeling reaction near neutral pH.

Thiol-modifying Dyes

Proteins have fewer thiol (or sulfhydryl, SH) groups than they do amine groups. Cysteines are the only thiol-bearing amino acids in proteins, and the frequency of cysteines is half the frequency of lysines. As a result, thiol-conjugated dyes are useful tools for detecting protein-protein interactions, measuring protein-ligand binding, and analyzing conformational changes within peptides. The latter is commonly achieved by modifying two thiols on a protein (either naturally occurring or introduced cysteines) with a fluorescence resonance energy transfer (FRET) donor and a FRET acceptor dye, respectively. Subsequent FRET analysis can reflect conformational change with respect to variables of interest.

The efficiency with which a particular cysteine is modified depends on both its surrounding environment within the protein and on the polarity of the reactive dye. By exploiting differential sensitivity to modification, as well as site-directed mutagenesis, thiol-reactive dyes can be added stoichiometrically and to specific sites on a protein,

enabling quantitative analysis. Thiol-reactive dyes can also be conjugated to oligonucleotides in which thiouridine has been incorporated, enabling the study of nucleic acid-ligand interactions.

One group of thiol-modifying dyes are thiolytes–heterocyclic nonfluorescent alkyl halides that react rapidly and preferentially with free thiol groups at physiological pH. Reactions yield photochemically stable fluorescent products which can be analyzed by HPLC/UV, gel electrophoresis, fluorescence microscopy, flow cytometry, or fluorometry. Examples of thiolytes are EMD Millipore's ThioGlo® and ThioLyte® dyes.

Chemically Crosslinked Dyes

Phycobiliproteins are highly efficient, light-gathering protein fluorochromes used most commonly for labeling antibodies for analysis by flow cytometry and fluorescence microscopy. These proteins feature very high quantum yields and high extinction coefficients, making them ideal for multicolor flow cytometry experiments. Because they do not contain chemically reactive groups, phycobiliproteins must be conjugated to a detection agent (typically either an antibody or to an affinity ligand, such as streptavidin) using a chemical crosslinker. EMD Millipore provides a large selection of antibodies and affinity ligands preconjugated to r-phycoerythrin, an intensely fluorescent protein produced by red algae.

Fluorescent Viability Stains and Intracellular Probes

Dyes can be used as reporters for intracellular conditions, such as pH, ion flux, apoptosis, autophagy, and vesicular transport. Intracellular probes are largely cell permeable; on the other hand, some viability stains, similar to the classic Trypan blue stain, only enter dead or dying cells. For quantitative assessments of viability, including assessments of apoptosis and overall cell health, multiparametric assays may provide faster, more accurate information.

For example, one multiparameter viability assay involves the use of two DNA intercalating fluorescent dyes in a single reagent (ViaCount® reagent). One of the dyes is membrane permeant and will stain all cells with a nucleus. The second dye only stains cells whose membranes have been compromised and are dying or dead. This combination allows for the discrimination of nucleated cells from those without a nucleus or debris, and live cells from dead or dying cells, resulting in both accurate cell concentration and viability results.

Table 4.3 illustrates the diversity of intracellular structures and processes that can be analyzed using fluorescent dyes.

Table 4.3 Some intracellular dyes and their corresponding applications

Intracellular Dye	Used to Detect:
SynaptoRed Reagent	Synaptic vesicles
Rhodamine 123	Mitochondria; Drug efflux
BCECF/AM	pH changes
Congo Red	Amyloid fibrils
DAF-2 DA	Nitric oxide (see below)
Dihydrorhodamine 123	Reactive oxygen species (ROS)
FLUO 3/AM	Calcium
JC-1	Mitochondrial potential
Propidium Iodide	Apoptotic cells and nuclei

Fluorescent calcium probes

Calcium plays a pivotal role in signal transduction and other cellular processes. Even minute changes in intracellular Ca^{2+} levels can have a major impact on cellular activities. Measurement of Ca^{2+} levels with fluorescent probes is one of the most sensitive techniques known. The method is based on the premise that these compounds display shifts in their excitation or emission spectra upon calcium binding.

In 1980, Roger Tsien introduced a new generation of Ca^{2+} chelators that have backbones similar to EGTA and are capable of chelating Ca^{2+} with high affinity and specificity. Molecules contain aromatic rings that interact electronically and sterically with the chelating backbone resulting in changes in their absorption and fluorescence properties upon calcium binding. The type of spectral changes which occur vary depending on the indicator used. It may be a shift in the absorbance spectra to a shorter wavelength with little change in the emission spectra (e.g., FURA 2); a shift in both absorbance and emission spectra to shorter wavelengths (e.g., INDO 1); or an increase or decrease in the quantum yield or efficiency of fluorescence (e.g., FLUO 3). These unique properties make these compounds highly suitable for use as sensitive probes for Ca^{2+}.

In general, the intensity of the fluorescent signal is dependent on the concentration of both the indicator and the ion. However, when Ca^{2+} binding results in a change in either the absorption or emission spectrum, it is possible to determine Ca^{2+} levels independently of the indicator concentration by using ratiometric techniques. In selecting the fluorescent probe appropriate for your application, it is important to consider both the expected intracellular calcium levels to be monitored and the

nature of the spectral changes associated with calcium binding. The maximal optical response occurs near the dissociation constant of the indicator. Hence, it is recommended that the dissociation constant be matched to the expected intracellular Ca^{2+} concentration.

Probes whose excitation spectra shift upon calcium binding are commonly used for fluorescent microscopy, while probes whose emission spectra shifts are better suited for use in flow cytometry. FLUO 3 is excited at longer wavelengths than many other probes, so it can be easily adopted for use in combination with caged compounds which release the active parent compound following irradiation with UV light.

Many of these probes are provided both as a lipophilic acetoxymethylester (AM) and as an alkali salt. The AM derivatives are cell-permeable. They are converted to free indicator by the hydrolytic activity of intracellular esterases. The alkali salt forms of these indicators are loaded into cells by microinjection or by permeabilization techniques. Microinjection is achieved by using pressure injection techniques with microelectrodes and ionophoresis electrodes. Chemical agents, such as digitonin, may also be used to permeabilize the cells.

Fluorescent Conjugated Antibodies

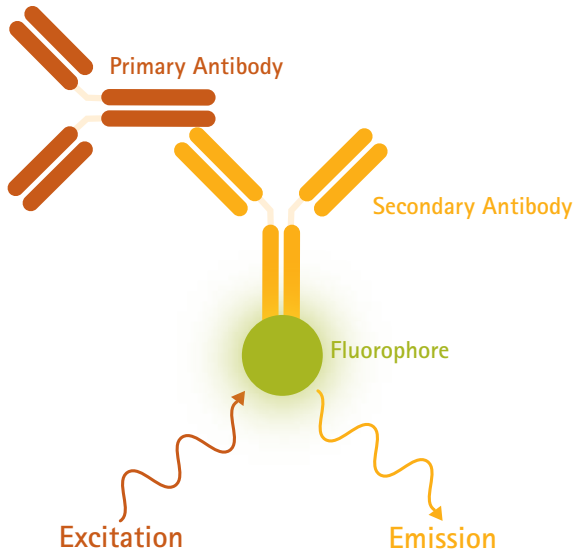


Figure 4.2
Fluorescent conjugated secondary antibody

Fluorescent antibody conjugates exhibit high specific fluorescence with minimal nonspecific staining. Fluorochromes such as rhodamine, phycoerythrin, FITC, and fluorescein are ideally suited for immunofluorescence assays. Labeled antibodies are useful both in direct and indirect detection methods and have applications in flow cytometry and immunofluorescence microscopy.

The performance of any conjugate in an immunofluorescence assay is largely dependent upon the purity and the concentration of the antibody involved. Antibodies with higher specificities exhibit low nonspecific staining. Suboptimal concentrations of antibody conjugates can lead to poor fluorescence intensity, whereas excessive conjugation can lead to either higher nonspecific binding or self quenching, depending upon the fluorochrome used.

EMD Millipore Calbiochem® Brand Dyes and Probes

Advantages

- Available in various grades and package sizes for greater flexibility
- Offer highly purified substances, to ensure reproducible results from the lab bench to production
- Manufactured in a highly controlled environment, ensuring the utmost purity and quality

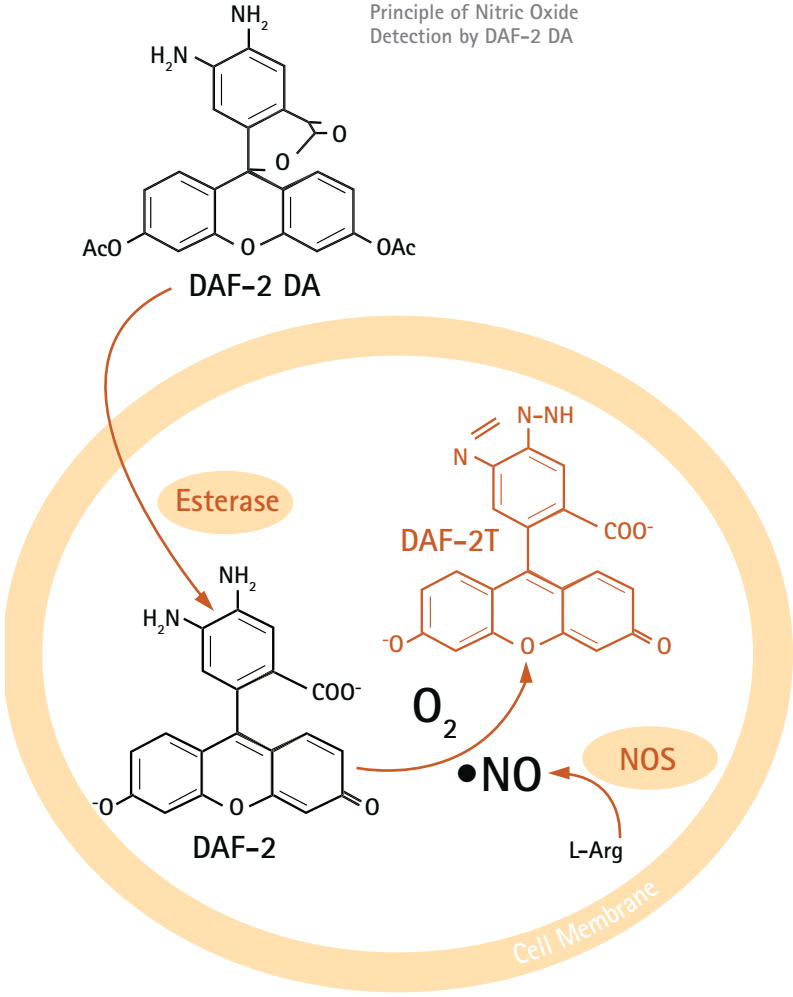
Available from: **www.emdmillipore.com**

DAF-2, common name: 4,5-Diaminofluorescein (Catalogue No. 251500-1MG)

A highly sensitive and specific fluorescent indicator for the direct detection of nitric oxide (•NO) *in vitro*. The relatively non-fluorescent DAF-2 reacts rapidly with NO in the presence of oxygen to yield the highly fluorescent triazolofluorescein compound. DAF can detect NO under neutral conditions (detection limit = 5 nM).

Nitric oxide (NO), a small biologically active messenger molecule, plays many important roles in human physiology that range from homeostasis to pathology. NO acts as a potent vasodilator, inhibits platelet aggregation, acts as an important neurotransmitter and mediates metabolism/blood flow coupling. Studies of the physiological roles of NO have been hampered by its extremely short half life and difficulty in measuring extremely small levels of intracellular and extracellular NO. Nitric oxide probes, such as 4,5-Diaminofluorescein Diacetate (DAF-2 DA), can be used for real-time bioimaging of NO with fine temporal and spatial resolution.

Principle of Nitric Oxide Detection by DAF-2 DA



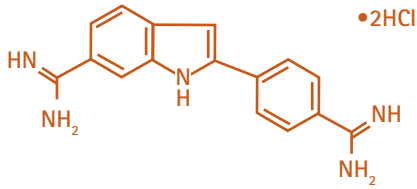
The sensitivity of DAF-2 is such that an increase in fluorescence intensity can be observed even in resting endothelial cells, reflecting the basal NO production. *In vitro*, studies have shown that DAF-2 does neither react with stable oxidized forms of NO such as NO_2^-, NO_3^-, nor with other reactive oxygen species such as O_2^-, H_2O_2, and $ONOO^-$. Other advantages of using DAF-2 for cellular imaging are its visible excitation wavelength that is less damaging to cells, and the fact that it is not subject to interference by the autofluorescence of common biological samples.

Probes for Nitric Oxide (NO) Research

Product	Description	Solubility	Size	Catalogue No.
4-AF DA	A useful negative control for the nitric oxide (NO) fluorescent indicator DAF-2 DA (Catalogue No. 251505). Unlike DAF-2 DA, 4-AF DA does not react with NO to form a fluorescent product.	DMSO	1 mg	121745
DAF-2	A highly sensitive and specific fluorescent indicator for the direct detection of nitric oxide (NO) *in vitro*. The relatively non-fluorescent DAF-2 reacts rapidly with NO in the presence of oxygen to yield the highly fluorescent triazolofluorescein compound (DAF-2T). Can detect NO under neutral conditions (detection limit = 5 nM). Ex. max: ~495 nm; Em. max: ~515 nm.	DMSO	1 mg	251500
DAF-2 DA	A cell-permeable derivative of DAF-2 (Catalogue No. 251500) that is hydrolyzed to DAF-2 by intracellular esterases. Can be used in fluorescence microscopy to measure real-time changes in NO levels *in vivo*. Ex. max: ~495 nm; Em. max: ~515 nm.	DMSO	1 mg	251505

DAPI, Dihydrochloride (Catalogue No. 268298-10MG)

A cell-permeable DNA-binding dye, DAPI binds preferentially to DNA rich in adenine and thymine. Used in flow cytometry for measuring nuclear DNA content or for sorting isolated chromosomes. Useful for microscopic detection of nuclei and nuclear DNA in normal and apoptotic cells. Can also be used to detect mycoplasma. A reversible inhibitor of S-adenosyl-L-methionine decarboxylase and diamine oxidase.



Fluoro-Jade® C (Catalogue No. AG325)

Fluoro-Jade® C, like its predecessors, Fluoro-Jade® and Fluoro-Jade® B, were found to stain all degenerating neurons, regardless of specific insult or mechanism of cell death. Therefore, the patterns of neuronal degeneration seen following exposure to either the glutamate agonist, kainic acid, or the inhibitor of mitochondrial respiration, 3-Nitropropionic acid (3-NPA), were the same for all of the Fluoro-Jade® dyes. However, there was a qualitative difference in the staining characteristics of the three fluorochromes. Specifically, Fluoro-Jade® C exhibited the greatest signal to background ratio, as well as the highest resolution. This translates to a stain of maximal contrast and affinity for degenerating neurons. This makes it ideal for localizing not only degenerating nerve cell bodies, but also distal dendrites, axons and terminals. The dye is highly resistant to fading and is compatible with virtually all histological processing and staining protocols. Triple labeling can be accomplished by staining degenerating neurons with Fluoro-Jade® C, cell nuclei with DAPI and activated astrocytes with GFAP immunofluorescence.

Appearance: Coffee brown to brick red powder.

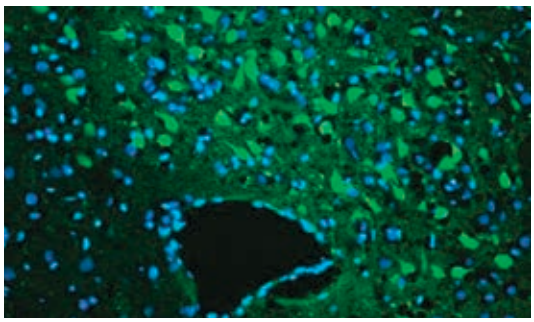
Molecular Weight: 823

Excitation Peak: 485 nm

Emission Peak: 525 nm

Filter System: Fluorescein / FITC

Solubility: Highly soluble in water and bases, moderately soluble in alcohol and weak acids.



Double exposure using ultraviolet and blue light excitation reveals blue DAPI labeled nuclei and green Fluoro-Jade® C (Catalogue No. AG325) positive cells and terminals in the dorsal thalamus following kainic acid exposure. Photomicrograph courtesy of Dr. Larry Schmued.

Black-Gold® II Stain (Catalogue No. AG400)

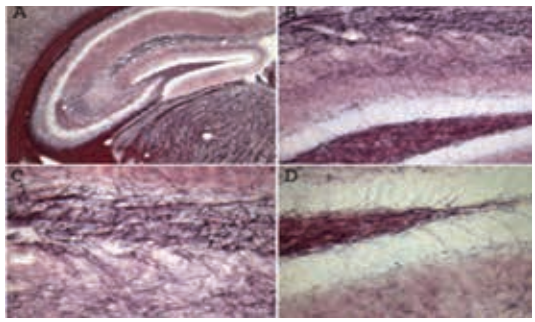
Black-Gold® II stain is an aurohalophosphate complex which stains specifically for myelin within the central nervous system. Black-Gold® II staining provides high resolution, high contrast, a short histochemical processing time, and high reproducibility. Black-Gold® II stain is a new and improved version of its predecessor, Black-Gold® stain (discontinued). The advantages of EMD Millipore's Black-Gold® II over Black-Gold® I stain are that it is more readily soluble, can be utilized at a higher concentration, produces a more uniform and consistent staining, takes less time to stain, and does not require post-staining intensification steps.

The use of Black-Gold® II stain is tailored to studies using formalin-fixed, non-solvent-processed tissue. The technique stains large myelinated tracts dark red-brown, while the individual myelinated fibers appear black. This novel tracer can be used to localize both normal and pathological myelin. Black-Gold® II stain can demonstrate and characterize specific myelin changes associated with exposure to diverse neurotoxicants including kainic acid, methamphetamine, acrylamide, domoic acid, 3-nitropropionic acid, Fluoro-Gold™ tracer and isoniazid.

Purity: No detectable amount of uncomplexed gold was found.

Illumination: Bright field or dark field.

Solubility: Freely soluble in water, saline, or dilute acids.

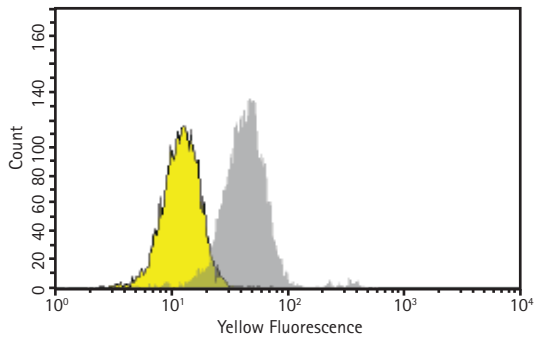


Examples of Black-Gold® II staining of cryosectioned mouse brain. (A) Hippocampus, thalamus and part of sensory motor cortex (5X magnification); (B) Hippocampus, 20X magnification; (C) Molecular layer at 40X magnification; (D) Dentate gyrus at 40X magnification. Note large bundles of myelin stained deep red while individual fibers appear black.

Milli-Mark™ Fluorescent Conjugated Antibodies

With the increasingly widespread use of flow cytometry and other methods of multiparameter cell analysis to identify and quantify biomolecules and cellular structures, many researchers have combined individual dyes and stains with fluorescent-conjugated specific antibodies optimized for flow cytometry. Milli-Mark™ fluorescent conjugated antibodies are specifically designed, optimized and validated for flow cytometry, exhibit superior signal-to-noise ratios, and eliminate the need for a secondary antibody.

To browse all Milli-Mark™ conjugated antibodies, visit:
www.millipore.com/cellularanalysis



Staurosporine-treated (yellow histogram) or untreated (grey histogram) Jurkat cells were stained using Milli-Mark™ pBcl2-(S70)-PE antibody (FCMAB140P) and analyzed using flow cytometry.

Key Products

Description	Size	Catalogue No.
Calcein-AM	1 mg	206700
MTT	1 g , 10 g	475989
Propidium Iodide	50 mg	537059

For more key dyes and stains from EMD Millipore, see page 73.

TECHNOLOGY HIGHLIGHT

Multiparameter Cell Analysis Using Benchtop Flow Cytometry

Fluorescent conjugated antibodies have made it possible to interrogate biological processes involving simultaneous, orchestrated changes in expression of multiple proteins by flow cytometry. Processing one cell at a time and measuring multiple proteins per cell simultaneously, a flow cytometer delivers richer, more predictive, and more quantitative research results compared to other methods of protein detection. EMD Millipore's guava easyCyte™ benchtop flow cytometry platform, paired with ready-to-use, validated FlowCellect™ kits and Milli-Mark™ conjugated antibodies, gives all researchers access to the power of flow cytometry, regardless of expertise or access to a core facility.

Key Benefits of guava easyCyte™ Systems:

- Choose from 1- or 2- laser formats designed for reliable single sample loading or fully automated 96-well plate handling
- Intuitive software with open and assay-specific formats provides results with minimal effort
- Easy to operate for precise and accurate detection of 5 to 8 parameters

Substrates

5

A substrate is a molecule that is the target of an enzyme's catalytic activity and yields an end product. Substrates that yield a detectable signal upon catalytic conversion are extremely useful for measuring enzymatic activity, either for studying the enzyme itself or in a reporter assay system to detect a coupled reaction. Substrates are widely used and, as such, qualify as essential biochemicals.

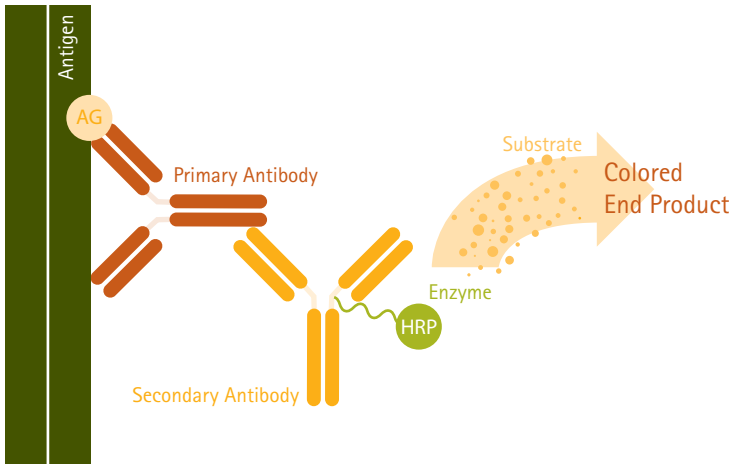


Figure 5.1
Example of a mechanism by which a chromogenic HRP substrate can be used in immunodetection.

Chemical Mechanisms of Common Substrates

Chromogenic Substrates

Upon enzymatic conversion, they produce an end product that absorbs light. The level of product generated can thus be monitored using a spectrophotometer. *p*-Nitroanilide (*p*NA) and thioester conjugated substrates are examples of chromogenic substrates.

Elastase substrate I, Colorimetric (Catalogue No. 324696), MeOSuc-Ala-Ala-Pro-Val-*p*NA is an example of a chromogenic protease substrate. The cleavage of the bond between Val and *p*NA will release free *p*NA that can be measured at 405 nm to 410 nm in a spectrophotometer.

MeOSuc-Ala-Ala-Pro-Val-*p*NA (no color)

MeOSuc-Ala-Ala-Pro-Val – *p*NA (colored)

Fluorogenic Substrates

They produce a fluorescent compound upon enzymatic conversion. Here the peptide is conjugated to a fluorogenic group such as AFC or AMC. The rate of release of the free AFC or AMC can be measured fluorometrically.

Elastase Substrate V, Fluorogenic (Catalogue No. 324740), MeOSuc-Ala-Ala-Pro-Val-AMC is an example of a fluorogenic protease substrate. The release of AMC can be measured upon protease cleavage of the substrate:

MeOSuc-Ala-Ala-Pro-Val-AMC (no fluorescence)

MeOSuc-Ala-Ala-Pro-Val – AMC (fluorescent)

Free AMC exhibits fluorescence. AMC has an excitation max: 380 nm and an emission max: 460 nm. A good fluorogenic compound should have a good Stokes shift (gap between the excitation and emission wavelengths).

Intramolecularly Quenched Substrates

These are almost always substrates of hydrolytic/proteolytic enzymes. A short peptide sequence separates a fluorescent donor group from an acceptor group, which acts as a quencher of fluorescence. Excitation energy is transferred from an excited fluorescent donor to the quenching acceptor via fluorescence resonance energy transfer. A cleavage of a peptide bond within the substrate leads to the separation of the donor-acceptor pair. This allows for a great increase in fluorescence, which can be monitored on a fluorometer. One of the most commonly used donor-acceptor pairs is EDANS (Donor) (5-[(2'aminoethyl)-amino]naphthalenesulfonic acid) and DABCYL (Acceptor) (4-[[4'-(dimethylamino] phenyl]azo]-benzoic acid). For example, Calpain-1 cleaves the following substrate:

H_2N-Glu(EDANS)-Pro-Leu-Phe ↓ Ala-Glu-Arg-Lys-(DABSYL)CO_2H

Cleavage of the peptide bond between Phe and Ala causes an increase in fluorescence (Excitation max: 335 nm; emission max: 505 nm).

Substrates for Biochemical Research

EMD Millipore offers a number of commonly used substrates: kinase substrates, phosphatase substrates, caspase substrates, MMP substrates, CHKtide, MBP substrate, uPA substrate, cathepsin and calpain substrates, chymotrypsin and granzyme B substrates. These are often provided in kits designed to assay enzyme activity.

Caspases are a family of cytosolic aspartate-specific cysteine proteases involved in the initiation and execution of apoptosis. EMD Millipore provides a number of activity assay kits for Caspases, both colorimetric and fluorometric. Each kit contains a substrate that is cleaved by the enzyme to generate a product that allows the enzyme activity to be measured.

Caspase Assay Kits

Description	Catalogue No.
Caspase 3/7 Assay Kit (Ac-DEVD-AMC Substrate)	17-367
Caspase 8 Colorimetric Activity Assay Kit, IETD	APT171
Caspase 10 Colorimetric Assay Kit, AEVD	APT176

For a complete listing of Caspase Assay Kits, visit: **www.emdmillipore.com**

Featured Products

EMD Millipore Calbiochem® Brand Substrates

Advantages

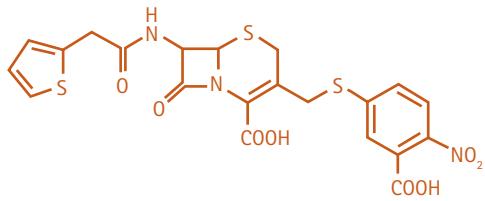
- Available for wide range of reactions
- Ensure ease of use to assay the proteolytic activity
- Available for a variety of applications
- Tested to the highest purity standards, ensuring reproducible results, lot-after-lot

Available from: **www.emdmillipore.com**

CENTA™ β-Lactamase Substrate (Catalogue No. 219475)

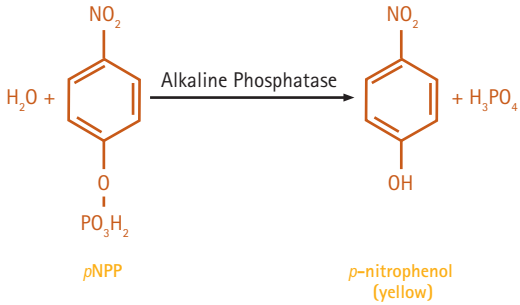
Chromogenic β-lactamase substrate for both gram-negative and gram-positive bacteria that is comparable to other chromogens (e.g., Nitrocefin). Hydrolysis of the β-lactam ring causes a color change from light yellow (λmax: 340 nm) to chrome yellow (λmax: 405 nm). Relatively unaffected by commonly used microbiological media and human serum. Stable in H_2O and in the presence of cysteine, mercaptoethanol and dimercaptopropanol.

Structure of CENTA™ β-Lactamase Substrate



*p*NPP (p-Nitrophenyl Phosphate) (Catalogue No. 4876)

This chromogenic substrate is used in phosphatase assays to detect the phosphatase activity by *p*NPP hydrolysis to *p*-nitrophenol. This yellow product can be quantitated by measuring its absorbance at 405 nm.



Luminata™ Chemiluminescent Substrates

Chemiluminescent HRP substrates are the most sensitive reagents for use in the detection of Western blots. Luminata™ Western ECL (electrochemiluminescence) substrates are a family of three, premixed detection reagents that cover a broad range of sensitivities, provide higher reproducibility by removing mixing steps, and are the most sensitive reagents in their class.

Luminata Western HRP Substrates are the most sensitive in their class

Comparison of different HRP substrates used during Western blotting. Western blots containing a 2-fold dilution series of A431 extract (ranging from 2–0.03 µg) were probed with either anti-PP2 , anti-STAT1, or anti-BRCA1 antibody followed by an appropriate secondary antibody. Blots were first visualized using the class-appropriate HRP substrate from the indicated supplier.

Key Products

Description	Size	Catalogue No.
Nitrocefin	5 g	484400
Novobiocin, Sodium Salt	10 g	491207
Proteasome Substrate III, Fluorogenic	5 g	539142
p-Nitrophenyl Phosphate	5 g	4876
X-GAL, OmniPur®	1 g	9630-100MG

For a complete listing of substrates from EMD Millipore, see page 76.

6 Other Essentials

In addition to indispensable products for research discussed in the previous chapters, there are a handful of products that are also very common and often used in labs.

Other Essentials include:

- Proteins – such as albumins are used as standards, for blocking, and as stabilizers.
- Small Molecules – are often used as inhibitors and activators.
- Amino Acids – are use in culture media, radiolabeling experiments, and more.
- Reducing Agents – are often employed in assays to prevent unwanted oxidation.
- Agarose – is a basic ingredient for gels in electrophoretic separation of DNA.

The sections below provide an overview of these research essentials.

Proteins

Albumins

Albumin is a ~66 kDa serum protein and the most abundant protein in mammalian blood plasma. It helps maintain osmotic pressure and serves as a carrier protein, delivering hormones, nutrients, fatty acids and other molecules to cells. Due to its several intramolecular disulfide bonds, albumin has high structural stability, enabling it to resist denaturation under many conditions. These properties, in combination with its high water solubility and very low isoelectric point (pI), it is not surprising that albumins are essential reagents in many biochemical experiments.

Applications:

- Cell culture: Because albumin binds and carries nutrients to cells and carries toxins away from cells, it is frequently added to cell cultures to promote overall cell health.
- Enzyme stabilizer: Used in molecular biology enzymatic reactions, such as restriction digests, to stabilize enzymes and prevent "star" activity
- Blocking: Because albumin can nonspecifically bind to various molecules and surfaces, it is used as a blocking agent to reduce nonspecific signals in immunodetection assays.
- Protein standard for quantitation of protein in experimental samples

Why is albumin sometimes called "Fraction V?" Plasma fractionation was invented in the 1940s during the search for stable plasma substitutes that retained the life-saving properties of plasma but were less labile. In the Cohn Fractionation procedure, blood proteins are separated into five fractions using different temperatures, biochemical conditions and organic solvents to maintain the proteins' biological activity. The fifth fraction contains albumin, leading to the name "Fraction V."

Table 6.1 Bovine serum albumins and their applications

Applications	Bovine Serum Albumins										
	Standard Albumin (Cat. No. 12659)	Protease Free (Cat. Nos. 126609, 2910)	Fatty Acid Free (Cat. No. 126575)	Endotoxin Free (Cat. No. 126579)	Nuclease Free (Cat. Nos. 126609, 126615, 2910)	Non-Denatured (Cat. No. 12660)	Crystalline (Cat. No. 12657)	Stabilizer Free (Cat. No. 126625)	Preservative Free (Cat. No. 126626)	RIA Grade (Cat. No. 126593)	DNP Conjugate (Cat. No. 324101)
Animal Perfusion Studies				●							
Antigen						◆	◆			◆	●
Binding and Transport Studies	◆		●								
Blocking Non-Specific Binding in ELISA and RIA Procedures	◆	◆	◆				◆			●	
Coating and Blocking of Plastic Surfaces										●	
Culture Media for Microbiology		◆	◆	●		◆	◆		●	◆	
Culture Media for Animal Cells		◆	◆	●		●			●	◆	
Growth Promoter in Hybridoma Procedures		◆	◆				◆			◆	
Hapten Carrier	◆					◆					
Molecular Biology					●						
Northern and Southern Blots					●						
Protein Base or Filter	◆	●					◆			◆	
Protein Standard, Molecular Weight						●	●			◆	
Stabilizer and Diluent in Blood Typing Reagents	●	◆									
Stabilizer and Diluent in ELISA and RIA Procedures	◆	◆	◆				◆	●		●	
Stabilizer and Diluent in Serology & Immunohematology	◆	●				◆	◆	◆		◆	
Immunoblotting										●	

● Designed specifically for this use.

◆ Suitable for this use.

Table 6.2 **Human serum albumins and their applications**

Applications	Human Serum Albumins			
	Standard Albumin (Cat. No. 12666)	Recrystallized (Cat. No. 126658)	Non-Denatured (Cat. No. 126654)	30% Solution (Cat. No. 12667)
Antigen		◆	◆	
Protein Base or Filler	◆	◆		◆
Blocking Non-Specific Binding in ELISA and RIA Procedures		◆		◆
Culture Media for Animal Cells		◆	◆	
Protein Standard, Molecular Weight		◆	◆	◆
Stabilizer and Diluent in Serology and Immunohematology	◆			

◆ Suitable for this use.

Lysozyme

Expressed in egg white, tears, and other secretions, lysozyme catalyzes the hydrolysis of N-acetylmuramide linkages in bacterial cell walls, thus acting as an antibacterial agent.

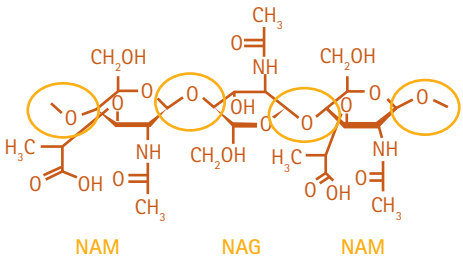


Figure 6.1

Lysozyme catalyzes the hydrolysis of the β(1-4) glycosidic bond between N-acetylglucosamine sugar (NAG) and N-acetylmuramic acid sugar (NAM) (circled in yellow).

Applications:

- Nucleic acid preparation
- Protein purification from inclusion bodies
- Plasmid preparation (to break down membranes and cell walls)
- Hydrolysis of chitin
- Hydrolysis of bacterial cell walls

The primary structure of egg white lysozyme is a single polypeptide chain of 129 amino acids. There are four pairs of cysteines that form disulfide bridges between positions 6 and 127, 30 and 115, 64 and 80, and 76 and 94 (counting from the N-terminal lysine). Because lysozyme is so easy to prepare from egg white and readily forms crystals, it was one of the first proteins for which structure was determined.

IPTG (Isopropyl-β-D-thiogalactopyranoside)

Expression of the three genes in the lac operon of *E. coli* is under the negative control of a repressor protein consisting of four identical 38.6 kDa subunits. IPTG, a free-flowing, non-hygroscopic carbohydrate, induces expression of the lac operon by allosterically interacting with the repressor molecule and causing a 300-fold reduction in the repressor's affinity for the operator sequence. The action of IPTG leads to the production of the lac operon gene products, which allows *E. coli* to utilize lactose as an energy source.

As a lac operon inducer, IPTG has been successfully used to increase the production of recombinant proteins produced in *E. coli*, such as somatostatin and the human insulin B chain. The IPTG-inducible lac operator/repressor system may also be used to produce regulated expression of transiently and stably transfected reporter genes. The regulatory elements of the lac operon have also been used to isolate conditional lethal mutants of poxviruses. These mutants are dependent on the presence of IPTG to produce infectious viruses.

Applications:

- Production of recombinant proteins in *E. coli*.
- Regulation of the expression of Lac operator/reporter genes that have been stably integrated into a mammalian chromosome.
- Recombinant viruses can be constructed such that the expression of specific target genes will be transcriptionally repressed unless IPTG is present.
- Induction of spontaneous lactose-utilizing mutants of *Vibrio vulnificus* and *Vibrio pelagius*.

Protease and Phosphatase Inhibitors and Inhibitor Cocktails

Proteases and Protease Inhibitors

Proper functioning of the cell requires a carefully controlled level of important structural, functional, and regulatory proteins. Proteins are continuously synthesized and degraded in plant and animal cells and a finely tuned balance exists between their rate of synthesis and breakdown that determines the concentration of any given protein. Protein degradation is an essential process whereby damaged, unwanted, or "used" proteins are continuously eliminated. Hence, in a laboratory setting, where the integrity of proteins is to be maintained during extraction, purification, and analysis, the use of protease inhibitors can minimize non-specific protein breakdown.

Protease inhibitor cocktails are important tools for scientists engaged in a variety of proteomics studies, including protein characterization, biomarker discovery, mapping of post-translational modifications, protein expression profiling, and the quantitative measurement of proteins. The addition of a protease inhibitor cocktail or protease inhibitor ensures the integrity of proteins for downstream analysis and further characterization of samples.

Protease inhibitors can be classified based on their mechanism of action:

- **Suicide inhibitors:** These are irreversible inhibitors that bind to the protease active site and are substrates for the protease. The enzyme catalyzes a reaction that results in the reaction product being covalently bound to the enzyme and preventing further protease activity. Examples: diisopropylfluorophosphate, pencillin, tosyl phenylalanyl chloromethyl ketone (TPCK), AEBSF
- **Transition state inhibitors:** These are reversible inhibitors that bind to the protease active site, mimicking the structure of the catalytic intermediate. Examples: phosphoramidon, bestatin, leupeptin
- **Protein protease inhibitors:** Serpins, which are proteins that irreversibly inhibit serine proteases, are an example. The protease cleaves the serpin, but at the point of the reaction in which the protease is still bound to the intermediate, the serpin causes a drastic conformational change in the protease that blocks further proteolysis. Examples: protein Z-dependent inhibitor; α2-macroglobulin, antithrombin
- **Chelating agents:** They chelate metal ions and thereby block activity of those enzymes which require these ions.

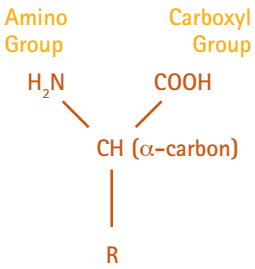
Table 6.3 **Protease inhibitors classified by their target class**

Protease type	Inhibitor examples	
Aspartyl proteases	Acetyl-pepstatin Pepstatin	
Cysteine proteases	Antipain Calpastatin Calpeptin Cathepsin inhibitors	Chymostatin Cystatin Leupeptin
Metalloproteases	1,10-phenanthroline Bestatin	EDTA/EGTA Phosphoramidon
Serine proteases	AEBSF Antichymotrypsin Antipain Antithrombin Antitrypsin Aprotinin Benzamidine	Bovine pancreatic trypsin inhibitor (BPTI) DFP Ecotin Leupeptin PMSF TLCK TPCK

Phosphatases and Phosphatase Inhibitors

Phosphorylation and dephosphorylation of proteins mediate signal transduction events that control a multitude of cellular processes such as cell division, cell proliferation, and apoptosis. Target proteins are phosphorylated by protein kinases that transfer a phosphate group to a specific protein, typically at serine, threonine, or tyrosine residues. The covalent attachment of a phosphoryl group changes the conformation of the protein and alters its ability to interact with a ligand. The phosphate group is removed by the action of protein phosphatases, which restore the protein to its original dephosphorylated state. For accurate characterization of the structure and function of phosphorylated proteins, it is critical to preserve their phosphorylation state during extraction from cell and tissue lysates. Phosphatase inhibitor cocktails are used to protect phosphoproteins from different families of phosphatases.

Amino Acids



Peptides and polypeptides are polymers of α-amino acids. There are 20 α-amino acids that make up all proteins of biological interest. The α-amino acids in peptides and proteins consist of a carboxylic acid (-COOH) and an amino (-NH2) functional group attached to the same tetrahedral carbon atom. This carbon is known as the α-carbon. The type of R-group attached to this carbon distinguishes one amino acid from another.

Some of the α-amino acids found in proteins are also involved in other functions in the body. For example, tyrosine is involved in the formation of thyroid hormones, and glutamate and aspartate act as neurotransmitters at fast junctions. Because amino acids are vital for all cell functions, they are essential biochemicals in many applications.

Applications:

- Preparation of defined cell culture media
- Radiolabeling cells for pulse-chase experiments to measure protein synthesis and degradation (metabolic labeling)
- *In vitro* transcription-translation to generate protein
- Preparing media for yeast screening assays, such as yeast two-hybrid or yeast three-hybrid assays, to detect intermolecular interactions
- Inclusion in nutritional supplements and fertilizers
- Industrial applications include the production of biodegradable plastics, drugs, and chiral catalysts
- The amino acid glycine is frequently used as a counterion in buffer systems to balance the charge of the buffer ion, particularly for gel electrophoresis

Amino acids exist in either D- or L- enantiomorphs or stereoisomers. The D- and L- refer to the absolute configuration of optically active compounds. With the exception of glycine, all other amino acids are mirror images that can not be superimposed. Most of the amino acids found in nature are of the L-type. Hence, eukaryotic proteins are always composed of L-amino acids although D-amino acids are found in bacterial cell walls and in some peptide antibiotics.

All biological reactions occur in an aqueous phase. Hence, it is important to know how the R-group of any given amino acid dictates the structure-function relationships of peptides and proteins in solution. The hydrophobic amino acids are generally located in the interior of proteins shielded from direct contact with water. Conversely, the hydrophilic amino acids are found on the exterior of proteins as well as in the active centers of enzymatically active proteins. It is the very nature of certain amino acid R-groups that allow enzyme reactions to take place. For example, histidine with an imidazole ring is found in the active site of several enzymes. This is due to the fact that the imidazole ring allows it to act as either a proton donor or acceptor at physiological pH. Equally significant is the ability of histidines in hemoglobin to buffer the H^+ ions (from carbonic acid ionization) in red blood cells. This property of hemoglobin facilitates the exchange of O_2 and CO_2 in tissues and lungs.

Table 6.4 Amino acids

Amino Acids	3-Letter Code	1-Letter Code	Molecular Weight	Pi Value	Empirical Formula
Alanine	Ala	A	89.09	6.00	$C_3H_7NO_2$
Arginine	Arg	R	174.20	11.15	$C_6H_{14}N_4O_2$
Asparagine	Asn	N	132.12	5.41	$C_4H_8N_2O_3$
Aspartic Acid	Asp	D	133.10	2.77	$C_4H_7NO_4$
Cysteine	Cys	C	121.15	5.02	$C_3H_7NO_2S$
Glutamine	Gln	Q	146.15	5.65	$C_5H_{10}N_2O_3$
Glutamic Acid	Glu	E	147.13	3.22	$C_5H_9NO_4$
Glycine	Gly	G	75.07	5.97	$C_2H_5NO_2$
Histidine	His	H	155.16	7.47	$C_6H_9N_3O_2$
Isoleucine	Ile	I	131.18	5.94	$C_6H_{13}NO_2$
Leucine	Leu	L	131.18	5.98	$C_6H_{13}NO_2$
Lysine	Lys	K	146.19	9.59	$C_6H_{14}N_2O_2$
Methionine	Met	M	149.21	5.74	$C_5H_{11}NO_2S$
Phenylalanine	Phe	F	165.19	5.48	$C_9H_{11}NO_2$
Proline	Pro	P	115.13	6.30	$C_5H_9NO_2$
Serine	Ser	S	105.09	5.68	$C_5H_7NO_3$
Threonine	Thr	T	119.12	5.64	$C_4H_9NO_3$
Tryptophan	Trp	W	204.23	5.89	$C_{11}H_{12}N_2O_2$
Tyrosine	Tyr	Y	181.19	5.66	$C_9H_{11}NO_3$
Valine	Val	V	117.15	5.96	$C_5H_{11}NO_2$

Cleland's Reagent

The inside of a cell has an overall reducing environment, meaning that functional groups are exposed to a ready source of electrons. Therefore, when carrying out biological and enzymatic reactions *in vitro*, chemical reducing agents are necessary to avoid unwanted oxidation of active species. Dithiothreitol (DTT), also known as Cleland's Reagent, is a water-soluble, protective reagent for sulfhydryl groups and is widely used for the study of disulfide exchange reactions of protein disulfides.

Physical Properties:

- Appearance: Non-hygroscopic, white powder
- Structure: $HS\text{-}CH_2\text{-}CH(OH)\text{-}CH(OH)\text{-}CH_2\text{-}SH$
- Empirical Formula: $C_4H_{10}O_2S_2$
- Molecular Weight: 154.3

Applications:

- Widely used as a protective agent for sulfhydryl groups
- Reducing agent for proteins
- Studies involving the regulation of gene expression
- May be used to distinguish a thiol-sensitive Type I aldolase and divalent metal sensitive Type II aldolase
- A protective agent against ionizing radiation in cells
- Enhances DNA polymerase activity
- By modifying one or more disulfide bonds on the NMDA receptors, DTT enhances synaptic activity in those areas of the brain where NMDA receptors are prominent
- May be used as an inducer of Gadd 153 mRNA

DTT readily permeates cell membranes and hence can protect protein sulfhydryls and restore enzyme activity lost by the oxidation of sulfhydryl groups. In human hepatoma HepG2 cells, DTT creates a reducing environment within the endoplasmic reticulum. This interferes with the formation of disulfide bonds that are essential for the proper folding of proteins. DTT is also known to be a reversible inhibitor of translational initiation in glial and pituitary tumor cells. By altering protein thiol-disulfide status, DTT causes the induction of a stress responsive gene gadd 153 mRNA. Addition of DTT to drug-treated cells restores their glutathione levels and may thereby reduce the toxicity of certain drugs.

Two fast-acting, stable reducing agents:

TCEP, Hydrochloride [Tris(2-carboxyethyl)phosphine, HCl] (Catalogue No. 580560-Y)

TCEP is considered to be a more potent, stable and effective alternative to DTT. It can reduce disulfide bonds even in strongly acidic environments (pH 1.5 to 4.5) where DTT is essentially ineffective. A comparison of the electrophoresis pattern, as well as quantitation using DTNB (Ellman's reagent), demonstrates that DTT was completely oxidized after 4 weeks and failed to reduce IgG to yield heavy and light chains.

- Water-soluble, odorless
- More stable and effective than DTT
- Acts over a broad pH range (pH 1.5 to 8.5)
- Fast acting
- More resistant to air-oxidation than DTT

BMS [Bis (2-mercaptoethyl)sulfone] (Catalogue No. 203325-1GM)

BMS is a water-soluble reagent useful for the reduction of native disulfide bonds in proteins, and is considered to be a superior reducing agent to DTT. Under non-denaturing conditions at pH 7, in certain samples, BMS has been shown to reduce accessible disulfide bonds 5 to 7 times faster than DTT.

- Water-soluble
- Low pK_a
- Stable in solution
- High reduction potential
- Acts faster than DTT
- Works even on less accessible disulfide bonds

50 mM TCEP, but not DTT, prevents IgG oxidation and aggregation in unattended samples. Electrophoresis pattern of human IgG using sample buffer with TCEP or DTT as the reducing agent. 2X sample buffers were left at room temperature for four weeks. Each day the tubes containing the buffer were left open for 15 – 30 minutes to mimic everyday use. Lane 1. Molecular weight markers. Lane 2, 3, 4, 5: four-week old sample buffer containing 10 mM TCEP, 50 mM TCEP, 10 mM DTT or 50 mM DTT, respectively.

Agarose

Agarose is a linear polysaccharide composed of alternating residues of D- and L-galactose that are joined by glycosidic linkages. It is obtained from agar, a natural product derived from algal cell walls. Agarose is used for a variety of life science applications especially in gel electrophoresis. Agarose forms an inert matrix (gel) utilized in separation techniques. Agarose gels are both porous and resilient and their molecular sieving properties enable the electrophoretic separation of charged macromolecules, such as DNA or RNA, according to size. Agarose has a lower resolution, but wider range of separation compared to polyacrylamide.

Applications:

- Electrophoretic separation of DNA derived from polymerase chain reaction (PCR), restriction digests, reverse transcription, etc.
- Electrophoretic separation of RNAs derived from total RNA preparations.
- Northern blotting.
- Isolation of DNA fragments for molecular cloning.

It is important to use the highest purity agarose for the most accurate electrophoretic separation and the most efficient recovery for molecular cloning. Lower grades of agarose can be contaminated with other polysaccharides, salts, and proteins. Such impurities can alter the gelling/melting temperature of agarose solutions or affect the ability to use the recovered nucleic acid sample in a post-electrophoresis application.

Key Products

Proteins

Description	Size	Catalogue No.
Albumin, Chicken Egg, 5X Crystalline	1 g	32467
	5 g	
Albumin, Mouse	25 mg	126674
Albumin, Rat	100 mg	126722
Lysozyme, Human Neutrophil	100 µg	440345
	1 mg	
Lysozyme, Chicken Egg White	1 g	4403
	5 g	
Lysozyme, Egg White, OmniPur®	5 g	5950-5GM
	10 g	5960-10GM
	1 kg	5975-1KG
rLysozyme™ Solution	300 KU	71110
	1200 KU	
	6000 KU	

OmniPur® Grade - DNase, RNase, and protease tested

Small Molecules and Inhibitors

Description	Size	Catalogue No.
IPTG, Dioxane-Free, High Purity*	1 g	420322
	10 g	
	100 g	
IPTG, OmniPur®	1 g	5800-1GM
	5 g	5810-5GM
	25 g	5815-25GM
	100 g	5820-100GM
IPTG, Animal-Free, High Purity	5 g	420291
	100 g	
Protease Inhibitor Cocktail Set III, EDTA-Free	1 mL	539134-1 ML
Protease Inhibitor Cocktail Set III, EDTA-Free	set	539134-1 SET
Phosphatase Inhibitor Cocktail Set II	set	524625-1 SET

Amino Acids

Description	Size	Catalogue No.
Glycine, OmniPur®	5 kg	4840-5KG
D-Cycloserine	5 g	239831-5GM
L-Selenomethionine	1 g	561505-1GM
L-Histidine, OmniPur®	5 kg	5450-5KG
L-Arginine, HCl	1 kg	181003-1KG

Reducing Agents

Description	Qty/Pk	Catalogue No.
Dithiothreitol, ULTROL® Grade*	1 g	233153
	10 g	
Dithiothreitol*	1 g	233155
	10 g	
REDUCTACRYL™ Reagent (Immobilized Cleland's Reagent)	1 g	233157
Dithiothreitol, OmniPur®	5 g	3860-5GM
TCEP, HCL	1 g	580560-1GM
TCEP, HCL, OmniPur®	2 g	4.85079
TCEP, Neutral	1 mL	580561-1ML
2-Mercaptoethanol, OmniPur®	100 mL	6010-100ML

Agarose

Description	Size	Catalogue No.
Agarose, OmniPur®	500 g	2125-500GM
Agarose, PCR Plus, OmniPur®	100 g	2010-100GM
Agarose, Low Melting , OmniPur®	100 g	2070-100GM
Agarose, type I, Molecular Biology Grade	100 g	121853-100GM
Agarose, Super Fine Resolution, OmniPur®	250 g	2082-250GM

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Description	Catalogue No.
Glutathione, Oxidized, Free Acid	3542
AEBSF, HCl	101500
BSA, Fraction V, Fatty Acid-Free	126575
BSA, Fraction V, RIA and ELISA Grade	126593
BSA, Fraction V, Fatty Acid, Nuclease and Protease-Free	126609
AMPK Inhibitor, Compound C	171260
Bisindolylmaleimide I	203290
Deoxycholic Acid	264101
ERK Inhibitor II *FR180204*	328007
HEPES, Free Acid, ULTROL Grade	391338
JNK Inhibitor II (SP600125)	420119
IPTG, Animal-Free, High Purity	420291
LY 294002	440202
MIF Antagonist, ISO-1	475837

Description	Catalogue No.
MES, Free Acid, ULTROL® Grade	475893
MOPS, Free Acid, ULTROL® Grade	475898
Myeloperoxidase, Polymorphonuclear Leukocytes	475911
n-Octyl-β-D-glucopyranoside	494459
PD98059	513000
Protease Inhibitor Cocktail Set III	539134
Proteinase K, *Tritirachium album*	539480
Rapamycin	553210
Rac1 Inhibitor	553502
SB 203580	559389
L-Selenomethionine	561505
Staurosporine, *Streptomyces* sp.	569397
Y-27632	688000

ULTROL® Grade - Uniform particle size and uniform solubility

7 Product Listing

Antibiotics

Product Name	Description	Sizes	Purity (≥)	Molecular Weight (g/mole)	CAS No.	Catalogue No.
Actinomycin D, 7-Amino-	DNA intercalator and has antibacterial properties. Exhibits growth-inhibitory activity against certain leukemias and sarcomas. Useful in distinguishing early apoptotic cells, which have lost membrane integrity, from apoptotic and live cells. Has been used in DNA analysis and as a probe for chromosome structure and function.	1 mg	≥85% by HPLC	1270.4	7240-37-1	129935
Actinomycin D, *Streptomyces* sp.	Anti-neoplastic antibiotic. Inhibits DNA-primed RNA polymerase by complexing with DNA via deoxyguanosine residues. Acts as a competitive inhibitor of serine proteases.	5 mg, 1 set	≥98% by HPLC	1255.5	50-76-0	114666
Ampicillin, Sodium , Sterile	A β-lactam antibiotic that inhibits bacterial cell-wall synthesis. Active against Gram-negative bacteria. Inactivated by lactamases. Suitable for use in research that uses ampicillin-resistant plasmids.	10 mL	NA	371.4	69-52-3	171257
Ampicillin, Sodium , Sterile, Tissue Culture Grade	A β-lactam antibiotic that inhibits bacterial cell-wall synthesis. Sterilized by γ-irradiation. Potency: ≥ 845 µg/mg.	20 mL	NA	371.4	69-52-3	171255
Ampicillin, Sodium Salt	A β-lactam antibiotic that inhibits bacterial cell-wall synthesis. Active against Gram-negative bacteria. Inactivated by lactamases. Suitable for use in research that uses ampicillin-resistant plasmids.	25 g	≥90% (anhydrous)	371.4	69-52-3	171254
Bafilomycin A1, *Streptomyces griseus*	A macrolide antibiotic that acts as a specific inhibitor. A valuable tool for distinguishing among different types of ATPases. Blocks lysosomal cholesterol trafficking in macrophages and is known to interfere with pH regulation in brain cells. Exhibits cytotoxic effects on a number of cell lines in a cell viability assay.	10 µg. 1 set	≥90% by HPLC	622.8	88899-55-2	196000
Benzalkonium Chloride	Cationic surface-active agent and germicide. Its effects are potentiated in the presence of di-n-butylesters, such as diisobutyladipate.	250 mL	NA	340	8001-54-5	198901
Blasticidin S, HCl, *S. griseochromogenes*	Nucleoside antibiotic that specifically inhibits protein synthesis in both prokaryotes and eukaryotes. Suitable for use as a dominant selectable marker in conjunction with blasticidin S resistant plasmids.	25 mg	≥98% by HPLC	458.9	589205	203350
Blasticidin S, *Streptomyces* sp.,	Nucleoside antibiotic that specifically inhibits protein synthesis in both prokaryotes and eukaryotes. Suitable for use as a dominant selectable marker in conjunction with blasticidin S resistant plasmids.	10 mL	NA	458.9	589205	203351
BLEOCIN®, *Streptomyces verticillus*, Cell Culture Tested	A unique antibiotic of the bleomycin family that is toxic to eukaryotic and prokaryotic cells. BLEOCIN™ Antibiotic cleaves double-stranded DNA and inhibits DNA synthesis, resulting in cell death. Can be used for selection of recombinant clones in a variety of cell types transfected with vectors containing a ble resistance marker. Copper content: ≥3.5%. Potency: ≥750 µg/mg.	10 mg 100 mg 250 mg	≥85% by HPLC	1613.5	55658-47-4	203408
BLEOCIN®, *Streptomyces verticillus*, Sterile	A unique antibiotic of the bleomycin family that is toxic to eukaryotic and prokaryotic cells. It cleaves double-stranded DNA and inhibits DNA synthesis, resulting in cell death. Can be used for selection of recombinant clones in a variety of cell types transfected with vectors containing a ble resistance marker.	10 mL	NA	1613.5	55658-47-4	203410

Antibiotics

Product Name	Description	Sizes	Purity (≥)	Molecular Weight (g/mole)	CAS No.	Catalogue No.
Bleomycin Sulfate, *S. verticillus*	Mixture of cytotoxic glycopeptides that inhibits DNA synthesis by reacting with DNA and causing strand scission. Acts by generating hydroxyl free radicals which cleave N-glycosidic bonds and cause base elimination (mainly T) and phosphodiester bond scission. An anti-neoplastic agent. Bleomycin has been used in the treatment of squamous cell carcinomas, testicular tumors, and malignant lymphomas.	10 mg	≥90% by HPLC (A2 + B2)	2927.17	9041-93-4	203401
Carbenicillin, Disodium Salt	Synthetic penicillin derivative for ampicillin-resistant plasmids. Blocks bacterial cell wall formation. Effective against Gram-negative bacteria.	250 mg	≥85% (USP)	422.4	4800-94-6	205805
Cefotaxime, Sodium Salt	Potent β-lactamase-resistant antibiotic of cephalosporin class. Active against Gram-positive and Gram-negative organisms, including Gram-negative anaerobes. Inhibits cell wall synthesis.	2 g	≥91% by HPLC (dry basis)	477.4	64485-93-4	219380
Cerulenin, *Cephalosporium caerulens*	An antifungal antibiotic that inhibits sterol and fatty acid biosynthesis. In fatty acid synthesis, reported to bind in equimolar ratio to β-keto-acyl-ACP synthase. In sterol synthesis, inhibits HMG-CoA synthetase activity. Also shown to inhibit feeding and induce dramatic weight loss in mice.	5 mg	≥98% by TLC	223.3	17397-89-6	219557
Chetomin, *Chaetomium* sp.	A cell-permeable dithiodiketopiperazine antimicrobial agent that disrupts the CH1 domain structure of p300, an important mediator of tumor hypoxia adaptation, and prevents the association of p300 with CH1-interacting proteins, such as HIF-1α and STAT2.	1 mg	≥97% by HPLC	710.9	1403-36-7	220320
Chloramphenicol	Synthetic bacteriostatic antibiotic that inhibits the translation of RNA by blocking the peptidyltransferase reaction on ribosomes.	100 g 500 g	≥97% by Assay	323.1	56-75-7	220551
Chromomycin A3	Antitumor antibiotic that inhibits RNA synthesis. A cell-permeable fluorescent dye that can be used with Bisbenzimide to distinguish chromosomes by their total DNA content and DNA base composition. It also interacts with DNA by binding to G:C pairs of helical DNA but does not intercalate.	10 mg	≥90% by HPLC	1183.3	7059-24-7	230752
Cycloheximide	An antifungal antibiotic that inhibits protein synthesis in eukaryotes but not prokaryotes.	1 g 5 g	≥90% by assay	281.3	66-81-9	239763
Cycloheximide, High Purity	Antifungal antibiotic that inhibits protein synthesis in eukaryotes but not in prokaryotes. Interacts directly with the translocase enzyme, interfering with the translocation step.	100 mg	≥98% by HPLC	281.3	66-81-9	239764
Erythromycin, *Streptomyces erythreus*	An antibacterial agent. Inhibits bacterial protein synthesis by binding to the 23S RNA in 50S ribosome.	5 g 25 g	≥85% by HPLC	733.9	114-07-8	329815
G418 Sulfate, Cell Culture Tested	Aminoglycoside related to gentamycin. Inhibits prokaryotic and eukaryotic protein synthesis. Toxic to bacteria, yeast, protozoans, helminths, higher plant, and mammalian cells. Widely used in the selection of eukaryotic expression vectors carrying the bacterial genes.	250 mg 500 mg 1 g 5g 25 g 100 g	≥98% by TLC	692.7	108321-42-2	345810
G 418 Sulfate, Cell Culture Tested	Sterile-filtered solution of G418 provided as 50 mg/mL active antibiotic.	10 mL 20 mL 50 mL	≥98% by TLC	692.7	108321-42-2	345812
Hygromycin B In PBS, Cell Culture Tested	Sterile liquid formulation of Catalogue No. 400050.	5 mL 20mL 50 mL	≥85% by HPLC and TLC	527.5	31282-04-9	400052

Antibiotics

Product Name	Description	Sizes	Purity (≥)	Molecular Weight (g/mole)	CAS No.	Catalogue No.
Hygromycin B, In 25 mM HEPES, Cell Culture Tested	Sterile liquid formulation of Catalogue No. 400050	5 mL 20 mL	≥90% by HPLC	527.5	31282-04-9	400053
Hygromycin B, *Streptomyces* sp.	Unique aminoglycoside antibiotic that inhibits the growth of prokaryotic (bacteria) and eukaryotic microorganisms (yeasts) and mammalian cells. Inhibits protein synthesis at the translocation step on the 70S ribosome and causes misreading of the mRNA.	100 KU 1 MU 5 MU 10 MU	≥85% by HPLC	527.5	31282-04-9	400051
Hygromycin B, *Streptomyces* sp., Cell Culture Tested	Unique aminoglycoside antibiotic that inhibits the growth of prokaryotic (bacteria) and eukaryotic microorganisms (yeasts) and mammalian cells. Inhibits protein synthesis at the translocation step on the 70S ribosome and causes misreading of the mRNA. Working concentration: 50 µg/mL - 1 mg/mL for mammalian cell selection.	100 mg 500 mg 1 g 5 g	≥90% by HPLC and TLC	527.5	31282-04-9	400050
Kanamycin Sulfate, *Streptomyces S. kanamyceticus*	Contains more than 98% kanamycin A. An aminoglycoside antibiotic effective against Gram-positive and Gram-negative organisms. Inhibitor of protein biosynthesis that acts on the 30S ribosome, causing misreading of the genetic code. May cause renal damage and ototoxicity.	25 g	NA	582.6	25389-94-0	420311
Kanamycin Sulfate, *S. kanamyceticus*	An aminoglycoside antibiotic effective against Gram-positive and Gram-negative organisms. Inhibitor of protein biosynthesis that acts on the 30S ribosome, causing misreading of the genetic code. May cause renal damage and ototoxicity.	20 mL	NA	582.6	25389-94-0	402412
Kanamycin Sulfate, *S. kanamyceticus*, Cell Culture Tested	An aminoglycoside antibiotic effective against Gram-positive and Gram-negative organisms. Inhibitor of protein biosynthesis that acts on the 30S ribosome, causing misreading of the genetic code. May cause renal damage and ototoxicity.	5 g 25 g	NA	582.6	25389-94-0	420411
Mitomycin C, *S. caespitosus*	Antibiotic and carcinostatic agent. Inhibits DNA synthesis by cross-linking DNA at guanine and adenine residues; disrupts base pairing. Induces apoptosis in gastric cancer cells. Blocks the cell cycle at the G2 phase. Potency: ≥970 µg/mg.	10 mg	≥95% by HPLC	334.3	50-07-7	475820
Mitomycin C, *Streptomyces caespitosus*, Carrier-Free	Antibiotic and carcinostatic agent. Inhibits DNA synthesis by cross-linking DNA at guanine and adenine residues; disrupts base pairing. Forms covalent adducts into deoxyguanine and guanine under acidic conditions. Induces apoptosis in gastric cancer cells. Blocks the cell cycle at the G2 phase.	2 mg	≥95% by HPLC	334.3	50-07-7	47589
Neomycin Sulfate	An aminoglycoside antibiotic that inhibits translation by binding to the small subunit of prokaryotic ribosomes. Recently shown to inhibit angiogenin-induced angiogenesis.	25 g	NA	908.9	1405-10-3	4801
Neomycin Sulfate, Tissue Culture Grade	Sterilized by γ-irradiation. An aminoglycoside antibiotic that inhibits translation by binding to the 30S ribosomal subunit.	20 mL	NA	908.9	1405-10-3	480100
Oxytetracycline, Hydrochloride	An antimicrobial agent. A member of the tetracycline family that inhibits bacterial protein synthesis by binding to the 30S ribosomal subunit.	10 g	≥97% by assay	496.9	2058-46-0	500105
Pen/Strept/Ampho B Soln. (100X), Tissue Culture Grade	Contains 10,000 units/mL Penicillin G, Potassium Salt, 10,000 µg/mL Streptomycin Sulfate, *Streptomyces* sp. and 25 µg/mL Amphotericin B, *Streptomyces* sp. Spectrum: bacteria, fungi, and yeast.	20 mL	NA	NA	NA	516104
Penicillin G, Potassium Salt	Antibiotic effective against Gram-positive bacteria. Blocks cell wall formation in growing cells, resulting in accumulation of uracil nucleotides of muramic acid peptides. Acts at the final stage of cell wall synthesis and prevents cross-linking of peptidoglycan strands.	100 mu	NA	372.5	113-98-4	5161

Antibiotics

Product Name	Description	Sizes	Purity (≥)	Molecular Weight (g/mole)	CAS No.	Catalogue No.
Penicillin/ Streptomycin, 100X, Tissue Culture Grade	A mixture of penicillin and streptomycin, supplied as 100X concentrated. Sterilized by γ-irradiation. Spectrum: Gram-positive and Gram-negative bacteria. When reconstituted in 20 mL of sterile deionized water contains 10,000 units/mL Penicillin G and 10 mg/mL. Streptomycin Sulfate.	20 mL	NA	NA	NA	516106
Polymyxin B Sulfate	An antibiotic that inhibits phospholipid-sensitive Ca^{2+} dependent protein kinases. Effective against Gram-negative bacteria.	500 mg 5 g	NA	200.36	1405-20-5	5291
Polymyxin B Sulfate, Sterile-Filtered	An antibiotic that inhibits phospholipid sensitive Ca^{2+} dependent protein kinase. Mixture of polymyxin B1 sulfate and polymyxin B2 sulfate. Binds to cell wall and makes it more permeable, causing fluid uptake. Effective against Gram-negative bacteria.	20 mL	NA	1385.61	1405-20-5	420413
Puromycin, DiHCl	Protein synthesis inhibitor. Inhibits translation by causing premature release of nascent polypeptide chains. Induces DNA fragmentation in thymocytes and in human HL-60 leukemia cells.	25 mg 100 mg	≥98% by TLC	544.4	58-58-2	540222
Puromycin, DiHCl, Cell Culture-Tested	Protein synthesis inhibitor. Inhibits translation by causing premature release of nascent polypeptide chains. Induces DNA fragmentation in thymocytes and in human HL-60 leukemia cells.	25 mg 100 mg	≥98% by TLC	544.4	58-58-2	540411
Rifampicin	Antibiotic that specifically inhibits DNA-dependent RNA polymerase in bacteria by forming an inactive complex. Does not affect mammalian RNA polymerase. Inhibits transcription by preventing the initial transcription complex from entering the elongation mode.	5 g	≥97% by HPLC (dry basis)	823	13292-46-1	557303
Spectinomycin, Dihydrochloride, Pentahyd	A broad spectrum aminoglycoside antibiotic that contains two glucose moieties. Footprint studies indicate that spectinomycin exerts regional effects on ribosomal structure.	10 g	NA	495.4	22189-32-8	567570
Streptozotocin	An N-nitroso-containing diabetogenic compound that acts as a nitric oxide donor in pancreatic islets. An antibiotic effective against Gram-negative bacteria. Causes DNA alkylation and DNA strand breaks in pancreatic islet cells.	250 mg 1 g	≥95% by HPLC	265.2	18883-66-4	572201
Tetracycline, HCl	Broad-based antibiotic agent that blocks protein synthesis by inhibiting binding of aminoacyl tRNA to the A-site of ribosomes. Induces cold shock-response and enhances P450 expression in bacteria.	10 g 25 g 50 g	NA	480.9	64-75-5	58346
Thiostrepton	A thiazole-containing peptide antibiotic that inhibits protein synthesis by preventing binding of GTP to 50S ribosomal subunit. The thiostrepton-resistant gene is also commonly used as a selective marker for recombinant DNA/plasmid technologies.	1 g 10 g	≥98% by TLC	1664.8	1393-48-2	598226
Tobramycin, Free Base	Aminoglycoside antibiotic active against Gram negative bacteria. Inhibits myeloperoxidase-dependent oxidant cell injury.	100 mg	NA	467.5	32986-56-4	614005
Triclosan	A potent antimicrobial agent that inhibits bacterial fatty acid synthesis by selectively targeting FabI encoded enoyl-acyl carrier protein (ACP) reductase. A slow-binding inhi	1 g	≥97% by HPLC	289.6	3380-34-5	647950
Tunicamycin, *Streptomyces lysosuperficus*	A nucleoside antibiotic that inhibits N-linked glycosylation and blocks the formation of N-glycosidic protein-carbohydrate linkages. Inhibits the expression of functional thrombin receptors on human T-lymphoblastoid cells.	10 mg 50 mg	≥95% by HPLC (A+B+C+D)	840.9	11089-65-9	654380
Vancomycin, HCl, *Streptomyces orientalis*	Amphoteric glycopeptide antibiotic. Inhibits bacterial mucopeptide synthesis by forming complexes.	250 mg	≥80% by HPLC	1485.7	1404-93-9	627850

Antifungals, Antiprotozoan, and Antivirals

Antifungals

Product Name	Description	Sizes	Purity (≥)	Molecular Weight (g/mole)	CAS No.	Catalogue No.
Amphotericin B, *Streptomyces* sp.	A polyene antifungal antibiotic that non-specifically induces loss of low molecular weight substances from cells. Binds to sterols and disrupts osmotic integrity of fungal membrane. Causes suppression of bone marrow progenitor cells.	100 mg	≥86% by HPLC	924.1	1397-89-3	171375
Clotrimazole	An antifungal agent that acts as a potent and specific inhibitor of the Ca^{2+}-activated K^+ channel (Gardos channel; IC_{50} = 650 nM). Prevents K^+ loss and dehydration of sickled erythrocytes.	100 mg	≥98% by TLC	344.8	23593-75-1	233230
Ketoconazole	An inhibitor of cytochrome P-450 in steroid biosynthesis. An antifungal agent that displays potent anti-metastatic, anti-neoplastic, and anti-psoriatic activities. Also acts as an inhibitor of 5-lipoxygenase (5-LO; IC_{50} = 26 µM) and thromboxane synthase activities.	50 mg	≥98% by HPLC	531.4	65277-42-1	420600
Nystatin, *S. noursei*, Sterile, Tissue Culture Grade	Sterilized by γ-irradiation. Antifungal antibiotic effective against yeast and mycoplasma. The complex contains 3 biologically active components, A1, A2, and A3. Reconstitute in 10 mL sterile deionized water to prepare a 5 mg/mL solution. Bioassay: ≥4400 units/mg.	50 mg	NA	926.09	1400-61-9	475921
Nystatin, *Streptomyces noursei*	A polyene antifungal antibiotic effective against yeast and mycoplasma. Induces loss of low molecular weight substances from cells with low selectivity. A cation-selective ionophore that exhibits selectivity for Na^+. Increases the activity of Na^+ - K^+ pump.	1 g 5 g	NA	926.1	1400-61-9	475914
Pimaricin, *Streptomyces chattanoogensis*	Antifungal polyene macrolide that is active in the cell membrane.	100 mg 1g	≥80% by HPLC	665.7	7681-93-8	527962
Polyoxin D, *Streptomyces cacaoi var. aso*	Specific inhibitor of fungal chitin synthetase.	1 mg	≥96% by HPLC	521.4	22976-86-9	529313

Antiprotozoan

Product Name	Description	Sizes	Purity (≥)	Molecular Weight (g/mole)	CAS No.	Catalogue No.
InSolution™ Sinefungin	An anti-leishmanial nucleoside antibiotic that acts as an S-adenosyl-L-methionine (SAM, AdoMet) methyltransferase-specific inhibitor.	2 mg	≥95% by HPLC	381.4	58944-73-3	567051

Antivirals

Product Name	Description	Sizes	Purity (≥)	Molecular Weight (g/mole)	CAS No.	Catalogue No.
Acyclovir	An antiviral nucleoside analog. Inhibits the proliferation of NIH 3T3 fibroblastic cells.	25 mg	≥98% by HPLC	225.2	59277-89-3	114798
Glycyrrhizin, Ammonium Salt	A triterpenoid saponin isolated from liquorice roots that displays antiviral and anti-tumor activities. Reported to block the growth of other viruses.	5g	≥98% by titration	840	53956-04-0	356780
Penciclovir	Active against several herpes viruses. Induces apoptosis effectively without exerting much genotoxicity.	100 mg	≥98% by HPLC	253.3	39809-25-1	516090
Ribavirin	A non-interferon-inducing, broad spectrum antiviral nucleoside. Reported to be active against RSV, HIV, HCV, and other infectious agents. Its metabolite, ribavirin-5′-phosphate, inhibits inosine monophosphate (IMP) dehydrogenase.	10 mg	≥99% by HPLC, ≥99% by TLC	244.2	36791-04-5	555580

Buffers

Product Name	Sizes	Purity (≥)	Molecular Weight (g/mole)	pK_a at 20 °C	Cas No.	Catalogue No.
100X TE Buffer SOL pH8, OmniPur®	1 L	NA	NA	NA	NA	8910-1L
10X MOPS, OmniPur®	500 mL	NA	209.26	7.2	1132-61-2	6350-500ML
10X PBS Dry Mix, OmniPur®	1 L	NA	NA	NA	NA	6508-1L
10X PBS Liquid Concentrate, OmniPur®	4 L	NA	NA	NA	NA	6505-4L
	1 L					6506-1L
10X TAE Buffer Liquid Concentrate, OmniPur®	20 L	NA	NA	NA	NA	8725-20L
	4 L	NA	NA	NA	NA	8720-4L
10X TBE Buffer Dry Mix, OmniPur®	1 Pack	NA	NA	NA	NA	8850-1PACK
10X TG Buffer, OmniPur®	4 L	NA	NA	NA	NA	9000-4L
10X TG-SDS Buffer, OmniPur®	4 L	NA	NA	NA	NA	9015-4L
10X PBS Liquid Concentrate., OmniPur®	4 L	NA	NA	NA	NA	6507-4L
10X TBE Buffer Liquid Concentrate, OmniPur®	20 L	NA	NA	NA	NA	8830-20L
	4 L					8820-4L
20X SSC Buffer Dry Mix, OmniPur®	1 Pack	NA	NA	NA	NA	8340-1PACK
20X SSPE Buffer Dry Mix, OmniPur®	1 Pack	NA	NA	NA	NA	8450-1PC
20X SSC Buffer, Liquid Concentrate, OmniPur®	4 L	NA	NA	NA	NA	8310-4L
20X SSPE Buffer, Liquid Concentrate, OmniPur®	4 L	NA	NA	NA	NA	8410-4L
25X TAE Buffer Dry Mix, OmniPur®	1 Pack	NA	NA	NA	NA	8750-1PC
5X TBE Buffer, Liquid Concentrate, OmniPur®	4 L	NA	NA	NA	NA	8800-4L
BES, Free Acid, ULTROL® Grade	25 g, 100 g	≥99% by titration (dry basis)	213.3	7.12	10191-18-1	391334
Bicine, ULTROL® Grade	100 g, 1 kg	≥99% by titration	163.2	8.35	150-25-4	391336
BIS-Tris Propane, ULTROL®Grade	100 g	≥98% by titration	282.4	6.8	64431-96-5	394111
BIS-Tris, ULTROL® Grade	100 g, 1 kg	≥99% by titration	209.2	6.5	6976-37-0	391335
CAPS, ULTROL® Grade	100 g, 1 kg	≥99% by titration	221.3	10.4	1135-40-6	239782
CHES, ULTROL® Grade	100 g	≥99% by titration	207.3	9.5	103-47-9	239779
HEPES, Free Acid, Molecular Biology Grade	25 g, 250 g	≥99% by alkalimetric assay	238.3	7.55	7365-45-9	391340
HEPES, Free Acid, ULTROL® Grade	100 g, 500 g, 1 kg, 5 kg	≥99% by titration (dry basis)	238.3	7.55	7365-45-9	391338
HEPES, Free Acid, ULTROL®, 1M Soln	100 mL, 500 mL	≥99% by titration (dry basis)	238.3	7.55	7365-45-9	375368
HEPES, Sodium Salt, ULTROL® Grade	100 g, 500 g, 1 kg	≥99% by titration (dry basis)	260.3	7.55	75277-39-3	391333
Imidazole, OmniPur®	50 g	≥99% by assay	68.08	7	288-32-4	5710-50GM
	500 g					5720-500GM
Imidazole, ULTROL® Grade	25 g, 100 g	≥99% by TLC and titration	68.08	7	288-32-4	4015
MES, Free Acid, ULTROL® Grade	100 g, 500 g, 1 kg	≥99% by titration (dry basis)	195.2	6.15	4432-31-9	475893
MES, OmniPur®	100 g	≥99% by assay	213.25	6.15	145224-94-8	6110-100GM
	1 kg					6120-1KG
MES, Sodium Salt, ULTROL® Grade	100 g, 1 kg	≥99% by titration (dry basis)	217.2	6.15	71119-23-8	475894
MOPS, Free Acid, Molecular Biology Grade	100 g	≥99% (Potentiometry)	209.3	7.2	1132-61-2	475922
MOPS, Free Acid, ULTROL® Grade	100 g, 500 g, 1 kg	≥99% by titration	209.3	7.2	1132-61-2	475898

OmniPur® Grade - DNase, RNase, and protease tested
ULTROL® Grade - Uniform particle size and uniform solubility

Buffers

Product Name	Sizes	Purity (≥)	Molecular Weight (g/mole)	pK_a at 20 °C	Cas No.	Catalogue No.
MOPS, OmniPur®	100 g	≥99% by assay	209.26	7.2	1132-61-2	6310-100GM
	1 kg					6320-1KG
MOPS, Sodium Salt, ULTROL® Grade	100 g, 1 kg	≥98% by titration (dry basis)	231.2	7.2	71119-22-7	475899
MOPS/EDTA Buffer, 10X Liquid Concentrate	100 mL	NA	NA	NA	NA	475916
HEPES, Free Acid, OmniPur®	100 g	≥99% by assay (dry basis)	238.3	7.55	7365-45-9	5310-100GM
	500 g					5320-500GM
	1 kg					5330-1KG
HEPES, Sodium Salt, OmniPur®	100 g	≥99% by assay (dry basis)	260.3	7.55	75277-39-3	5380-100GM
	1 kg					5390-1KG
PBS Tablets, OmniPur®	100/PK	NA	NA	NA	NA	6500-100TAB
	200/PK					6501-200TAB
PBS Tablets	1 ea - 10 tablets	NA	NA	NA	NA	524650
PBS-TWEEN® Tablets	1 ea- 10 tablets	NA	NA	NA	NA	524653
PIPES, Free Acid, ULTROL® Grade	100 g	≥98% by titration (dry basis)	302.4	6.8	5625-37-6	528131
PIPES, Sesquisodium Salt, ULTROL® Grade	100 g, 1 kg	≥98% by titration (dry basis)	335.3	7.2	100037-69-2	528132
PIPES, Sodium Salt, OmniPur®	250 g	≥99% by assay	335.37	6.10-7.50	100037-69-2	6910-1KG
	1 kg					6912-1KG
PIPPS	100 g	≥97% by titration (dry basis)	330.4	7.96	5625-56-9	528315
SSC Buffer, 20X Powder Pack, ULTROL®	2 Pack	NA	NA	NA	NA	567780
SSPE Buffer Tablets	1 ea	NA	NA	NA	NA	567782
TAE Buffer, 10X, Molecular Biology Grade	1 L	NA	NA	NA	NA	574797
TAPS, ULTROL® Grade	1 kg	≥98% by titration	243.2	8.4	29915-38-6	394675
TBE Buffer, 10X Powder Pack, ULTROL® Grade	2 Pack	NA	NA	NA	NA	574796

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Buffers

Product Name	Sizes	Purity (≥)	Molecular Weight (g/mole)	pK_a at 20 °C	Cas No.	Catalogue No.
TBS 20X Liquid Concentrate OmniPur®	4 L	NA	NA	NA	NA	8320-4L
TBS 20X Ready Pack Powder, OmniPur®	2 Pack	NA	NA	NA	NA	8325-2PACK
TE Buffer, 100X, Molecular Biology Grade	1 L	NA	NA	NA	NA	574793
TES, Free Acid, ULTROL® Grade	100 g, 1 kg	≥99% by titration (dry basis)	229.3	7.5	7365-44-8	39465
TG-SDS Buffer, 10X Powder Pack, ULTROL®	2 Pack	NA	NA	NA	NA	585207
Tricine, OmniPur®	500 g	≥99% by assay	179.17	8.15	1389475	9010-500GM
Tricine, ULTROL® Grade	100 g, 1 kg	≥99% by titration	179.2	8.15	1389475	39468
Triethylammonium Acetate, 1M Solution	1 L	NA	161.2	NA	5204-74-0	625718
TRIS 1M, pH 7.5, OmniPur®	100 mL	NA	121.1	8.1	77-86-1	9285-100ML
TRIS 1M, pH 8.0, OmniPur®	100 mL	NA	121.1	8.1	77-86-1	9290-100ML
TRIS 1M, pH 9.0, OmniPur®	100 mL	NA	121.1	8.1	77-86-1	9295-100ML
TRIS 1M, pH 10.0, OmniPur®	100 mL	NA	121.1	8.1	77-86-1	9299-100ML
TRIS Base, Molecular Biology Grade	500 g, 2.5 kg	≥99% by titration test (perchloric acid titration)	121.1	8.3	77-86-1	648310
TRIS Base, ULTROL® Grade	1 kg, 5 kg	≥99% by titration (dry basis)	121.1	8.3	77-86-1	648311
TRIS Buffer, 1M, pH 8.0, Molecular Biology Grade	100 mL	NA	121.1	8.1	77-86-1	648314
TRIS Buffer, 100 mM, pH 7.4, Molecular Biology Grade	100 mL	NA	121.1	8.1	77-86-1	648315
TRIS HCl, OmniPur®	500 g	≥99% by assay	157.6	8.3	1185-53-1	9310-500GM
	5 kg					9350-5KG
TRIS, HCl, Molecular Biology Grade	100 g, 1 kg	≥99% by titration	157.6	8.3	1185-53-1	648317
TRIS, HCl, ULTROL® Grade	250 g, 500 g, 1 kg	≥99% by titration	157.6	8.3	1185-53-1	648313
TRIS, OmniPur®	500 g	≥99% by assay	121.1	NA	77-86-1	9210-500GM
	5 kg	≥99% by assay	121.1	NA	77-86-1	9230-5KG
	25 kg	≥99% by assay	121.1	NA	77-86-1	9260-25KG

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Detergents

Product Name	Sizes	Classification	CMC value (Critical Micellar Concentration)	Catalogue No.
APO Detergent Set	1 Set	Non-Ionic Detergents	NA	178400
APO-10	1 g	Non-Ionic Detergents	4.6 mM	178375
APO-12	1 g	Non-Ionic Detergents	568 µM	178377
ASB ZWITTERGENT® Set	1 Set	Zwitterionic Detergents	NA	182753
ASB-14	25 g	Zwitterionic Detergents	NA	182750
ASB-14-4	1 g, 5 g	Zwitterionic Detergents	NA	182751
ASB-16	5 g, 25 g	Zwitterionic Detergents	NA	182755
ASB-C7BzO	1 g, 5 g	Zwitterionic Detergents	NA	182729
ASB-C8Ø	1 g, 5 g	Zwitterionic Detergents	NA	182730
Big CHAP	1 g	Non-Ionic Detergents	3.4 mM	200965
Big CHAP, Deoxy	250 mg, 1 g	Non-Ionic Detergents	1.1 - 1.4 mM	256455
BRIJ 35 Detergent, 30% Aqueous Solution	100 mL, 1 L	Non-Ionic Detergents	92 µM	203724
BRIJ 35 Detergent, Protein Grade, 10% Solution	50 mL	Non-Ionic Detergents	92 µM	203728
$C_{12}E_8$	1 g	Non-Ionic Detergents	110 µM	205528
$C_{12}E_8$, Protein Grade, Detergent, 10% Solution	1 Set	Non-Ionic Detergents	110 µM	205532
$C_{12}E_9$, Protein Grade, Detergent, 10% Solution	1 Set	Non-Ionic Detergents	80 µM	205534
CHAPS	1 g, 5 g, 10 g, 25 g	Zwitterionic Detergents	6 - 10 mM	220201
CHAPS, OmniPur®	10 g	Zwitterionic Detergents	6 - 10 mM	3050-10GM
	100 g	Zwitterionic Detergents	6 - 10 mM	3055-100GM
CHAPSO	1 g, 5 g	Zwitterionic Detergents	8 mM	220202
Chenodeoxycholic Acid, Sodium Salt	5 g	Ionic Detergents	NA	220411
CHES, OmniPur®	100 g	NA	NA	3100-100GM
Cholic Acid, Sodium Salt	50 g, 250 g	Ionic Detergents	9 - 15 mM	229101
Cholic Acid, Sodium Salt, ULTROL® Grade	1 g, 5 g	Ionic Detergents	9 - 15 mM	229102
CTAB, Molecular Biology Grade	100 g	Ionic Detergents	NA	219374
Cyclohexyl-n-hexyl-b-D-maltoside, ULTROL® Grade	1 g	Non-Ionic Detergents	560 µM	239775
DDMAB	5 g	Zwitterionic Detergents	4.3 mM	252000
Deoxycholic Acid, Sodium, ULTROL® Grade	5 g, 25 g	Ionic Detergents	2 - 6 mM	264103
Deoxycholic Acid, Sodium Salt	25 g, 100 g, 1 kg	Ionic Detergents	4 - 8 mM	264101
Detergent Variety Pack	1 Ea	Detergent Sets	NA	263458
Digitonin, High Purity	250 mg, 1 g, 5 g	Non-Ionic Detergents	NA	300410
ELUGENT® Detergent, 50% Solution	100 mL	Non-Ionic Detergents	NA	324707
GENAPOL® X-100, Protein Grade, Detergent, 10% Solution	50 mL	Non-Ionic Detergents	150 µM	345798

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Detergents

Product Name	Sizes	Classification	CMC value (Critical Micellar Concentration)	Catalogue No.
GENAPOL® X-100, Protein Grade, Detergent, 10% Solution	50 mL	Non-Ionic Detergents	60 - 150 µM	345796
Glycocholic Acid, Sodium Salt	1 g, 5 g	Ionic Detergents	7.1 mM	360512
Glycodeoxycholic Acid, Sodium Salt	5 g	Ionic Detergents	2.1 mM	361311
HECAMEG®	5 g	Non-Ionic Detergents	19.5 mM	373272
Lauroylsarcosine, Sodium Salt	5 g	Ionic Detergents	NA	428010
LPD-12	1 mg, 2 mg	Ionic Detergents	< 1 µM	437600
MEGA-9, ULTROL® Grade	5 g	Non-Ionic Detergents	19 - 25 mM	444930
n-Decanoylsucrose	1 g, 5 g	Non-Ionic Detergents	2.5 mM	252721
n-Decyl-β-D-maltopyranoside, ULTROL® Grade	1 g, 5 g	Non-Ionic Detergents	1.6 mM	252718
n-Dodecanoylsucrose	5 g	Non-Ionic Detergents	300 µM	324374
n-Dodecyl-β-D-glucopyranoside	1 g	Non-Ionic Detergents	130 µM	324351
n-Dodecyl-β-D-maltoside, ULTROL® Grade	1 g, 5 g, 25 g, 100 g	Non-Ionic Detergents	100-600 µM	324355
n-Hexyl-β-D-glucopyranoside	1 g	Non-Ionic Detergents	NA	376965
n-Nonyl-β-D-glucopyranoside	1 g	Non-Ionic Detergents	6.5 mM	488285
n-Octanoylsucrose	5 g	Non-Ionic Detergents	24.4 mM	494466
n-Octyl-β-D-glucopyranoside	1 g, 5 g, 25 g	Non-Ionic Detergents	20 - 25 mM	494459
n-Octyl-β-D-glucopyranoside, ULTROL® Grade	250 mg, 1 g, 5 g	Non-Ionic Detergents	20 - 25 mM	494460
n-Octyl-β-D-thioglucopyranoside, ULTROL® Grade	5 g	Non-Ionic Detergents	9 mM	494461
n-Octyl-β-D-maltopyranoside	1 g	Non-Ionic Detergents	19.5 mM	494465
NDSB Set	1 Set	Non-Detergent Sulfobetaines	NA	480012
NDSB-201	25 g, 250 g	Non-Detergent Sulfobetaines	NA	480005
NDSB-221	5 g, 25 g	Non-Detergent Sulfobetaines	NA	480014
NDSB-256	5 g, 25 g	Non-Detergent Sulfobetaines	NA	480010
NP-40 Alternative	100 mL, 500 mL, 1000 mL	Non-Ionic Detergents	50 - 300 µM	492016
NP-40 Alternative, Protein Grade, 10% Solution	50 mL, 500 mL	Non-Ionic Detergents	50-300 µM	492018
PLURONIC F-127, Protein Grade, 10% Solution	50 mL	Non-Ionic Detergents	NA	540025
Saponin	100 g	Non-Ionic Detergents	0.1% (w/v)	558255
SDS Solution 20%, OmniPur®	1 L	Ionic Detergents	7-10 mM	7991-1L
SDS Tablets	1 Ea	Ionic Detergents	7-10 mM	428029

OmniPur® Grade - DNase, RNase, and protease tested
ULTROL® Grade - Uniform particle size and uniform solubility

Detergents

Product Name	Sizes	Classification	CMC value (Critical Micellar Concentration)	Catalogue No.
SDS, 20% Solution (w/v)	200 mL	Ionic Detergents	7-10 mM	428018
SDS, Molecular Biology Grade	50 g, 500 g	Ionic Detergents	7-10 mM	428023
SOD Dodecyl Sulfate, OmniPur®	500 g	Ionic Detergents	7-10 mM	7910-500GM
	200 mL			7990-200ML
Sodium *n*-Dodecyl Sulfate	1 kg	Ionic Detergents	7-10 mM	428015
Sodium *n*-Dodecyl Sulfate, High Purity	25 g	Ionic Detergents	7 - 10 mM	428016
Taurocholic Acid, Sodium Salt, ULTROL® Grade	1 g, 5 g	Ionic Detergents	3 - 11 mM	580218
Taurocholic Acid, Sodium Salt	5 g, 25 g	Ionic Detergents	3 - 11 mM	580217
Taurodeoxycholic Acid, Sodium Salt	5 g, 50 g	Ionic Detergents	1 - 4 mM	580221
Tauroursodeoxycholic Acid, Sodium Salt	1 g, 5 g	Ionic Detergents	NA	580549
TRITON® X-100 Detergent	1 kg, 3 kg	Non-Ionic Detergents	200 - 900 µM	648462
TRITON® X-100 Detergent, Hydrogenated	10 g	Non-Ionic Detergents	250 µM	648465
TRITON® X-100 Detergent, Molecular Biology Grade	50 mL	Non-Ionic Detergents	200 - 900 µM	648466
TRITON® X-100, Hydrogenated, 10% Solution	10 mL	Non-Ionic Detergents	250 µM	648464
TRITON® X-100, Protein Grade, 10% Solution	50 mL	Non-Ionic Detergents	200 - 900 µM	648463
TRITON® X-114, Protein Grade, 10% Solution	50 mL	Non-Ionic Detergents	350 µM	648468
TRITON® X-100, OmniPur®	100 mL	Non-Ionic Detergents	200 - 900 µM	9400-100ML
	1L			9410-1L
	4 L			9440-4L
	20 L			9460-20L
TWEEN® 20 Detergent	250 mL	Non-Ionic Detergents	59 µM, HLB number 16.7	655205
TWEEN® 20 Detergent, Molecular Biology Grade	100 mL	Non-Ionic Detergents	NA	655204
TWEEN® 20, Protein Grade, Detergent, 10% Solution	50 mL	Non-Ionic Detergents	59 µM, HLB number 16.7	655206
TWEEN® 80, Protein Grade, Detergent, 10% Solution	50 mL	Non-Ionic Detergents	12 µM, HLB number 15	655207
TWEEN® 20, OmniPur®	1 L	Non-Ionic Detergents	59 µM	9480-1L
	4 L	Non-Ionic Detergents	59 µM	9485-4L
TWEEN® 80, OmniPur®	1 L	Non-Ionic Detergents	NA	9490-1L
	4 L	Non-Ionic Detergents	NA	9495-4L
ZWITTERGENT® 3-08 Detergent	5 g	Zwitterionic Detergents	330 mM	693019
ZWITTERGENT® 3-10 Detergent	5 g, 25 g, 100 g	Zwitterionic Detergents	25 - 40 mM	693021
ZWITTERGENT® 3-12 Detergent	5 g, 25 g	Zwitterionic Detergents	2 - 4 mM	693015
ZWITTERGENT® 3-14 Detergent	5 g, 25 g, 100 g	Zwitterionic Detergents	100 - 400 µM	693017
ZWITTERGENT® 3-16 Detergent	5 g, 25 g	Zwitterionic Detergents	10 - 60 µM	693023
ZWITTERGENT® Test Kit	1 Kit	Zwitterionic Detergents	NA	693030

OmniPur® Grade - DNase, RNase, and protease tested
ULTROL® Grade - Uniform particle size and uniform solubility

Product Name	Description	Excitation	Emission	Molecular Weight (g/mole)	Catalogue No.
2′,7′-Dichlorofluorescin Diacetate	Cell-permeable fluorogenic probe that is useful for the detection of reactive oxygen species (ROS) and nitric oxide (NO) and for the determination of the degree of overall oxidative stress.	502 nm	523 nm	487.3	287810
5,5′-Dithio-bis-(2-nitrobenzoic Acid), DTNB	Sulfhydryl reagent used to characterize reactive SH groups. Useful in photometric determination of thiols.	NA	NA	396.4	322123
5-Carboxyfluorescein Diacetate	Vital stain. Non-fluorescent, hydrophobic agent that permeates intact cell membranes. In the cell, it is enzymatically hydrolyzed to a hydrophilic agent, 5-Carboxyfluorescein that exhibits strong fluorescence.	494 nm	521 nm	460.4	216275
7-Amino-4-methylcoumarin	Reagent used to prepare fluorogenic 7-amido-4-methylcoumarin (AMC) based substrates for the detection of proteolytic enzyme activity. Useful as a reference standard in enzyme assays.	365-380 nm	430-460 nm	175.2	164545
Acridine Orange	A cell-permeable, cationic fluorescent dye that interacts with DNA and RNA by intercalation or electrostatic attractions.	502 nm (DNA-bound) 460 nm (RNA-bound)	525 nm (DNA-bound) 650 nm (RNA-bound)	301.8	113000
BCECF/AM	A variable mixture of cell-permeable ester derivatives of BCECF that are hydrolyzed by cytosolic esterases to yield intracellularly trapped indicator BCECF. Has been used to monitor intracellular pH changes in mammalian fibroblasts, gastric cells, lymphocytes, myocytes, and distal convoluted tubules.	~504 nm	~526 nm	880.7	216254
Bisbenzimide H 33258 Fluorochrome, Trihydrochloride	Membrane-permeable, adenine-thymine-specific fluorescent stain. Useful for staining DNA, chromosomes, and nuclei.	346 nm	460 nm	533.9	382061
Bisbenzimide H 33342 Fluorochrome, Trihydrochloride	Cell-permeable, adenine-thymine-specific fluorescent stain. Useful for staining DNA, chromosomes, and nuclei for fluorescence microscopy and flow cytometry applications.	346 nm	460 nm	561.9	382065
BTA-1	A fluorescent Thioflavin-T (Catalogue No. 596200) derivative that exhibits high affinity for amyloid deposits (Ki = 11 nM for Aβ 40). Crosses the blood brain barrier and displays up to 50-fold higher affinity than ThT (Catalogue No. 596200).	~380-480 nm	750 nm	240.3	203860
Calcein-AM	A cell-permeable, non-fluorescent, and hydrophobic compound, which is rapidly hydrolyzed by intracellular esterases releasing the membrane-impermeable, hydrophilic, and intensely fluorescent calcein. Can also be used for imaging mitochondrial permeability pore transition.	494 nm	517 nm	994.9	206700
Coelenterazine-h, Synthetic	A highly sensitive and a specific intracellular luminophore that is useful for measuring changes in levels of superoxide and Ca^{2+} in cells transfected with apoaequorin cDNA. Exhibits nearly 16-fold greater luminescence intensity.	NA	~466 nm	407.5	233903
Congo Red, High Purity	An amyloidophylic dye that specifically stains stacked β; sheet aggregates. Useful for staining and visual detection of amyloid deposits.	497 nm	614 nm	696.7	234610
DAF-2	A highly sensitive and specific fluorescent indicator for the direct detection of nitric oxide (NO) *in vitro*.	~495 nm	~515 nm	362.3	251500
DAF-2 DA	A cell-permeable derivative of DAF-2 (Catalogue No. 251500) that is hydrolyzed to DAF-2 by intracellular esterases. Can be used in fluorescence microscopy to measure real-time changes in NO levels *in vivo*.	~495 nm	~515 nm	446.4	251505

Product Name	Description	Excitation	Emission	Molecular Weight (g/mole)	Catalogue No.
DAF-FM	A photo-stable nitric oxide (NO) fluorescent indicator with a detection limit of ~3 nM. Reacts with NO in the presence of O_2, resulting in a triazolo-fluorescein analog (DAF-FM T) that exhibits about 160-fold greater fluorescence quantum efficiency.	~500 nm	~515 nm	412.4	251515
DAF-FM DA	A cell-permeable, photo-stable nitric oxide (NO) fluorescent indicator with a detection limit of ~3 nM. Intracellularly releases DAF-FM (Catalogue No. 251515) by the action of esterases.	~500 nm	~515 nm	496.4	251520
Dansyl Chloride	Reagent used for fluorescent labeling of proteins, N-terminal amino acids, and amines.	335 nm	500 nm	269.8	311155
DAPI, Dihydrochloride	Cell-permeable DNA-binding dye. Binds preferentially to DNA rich in adenine and thymine.	~359 nm	~461 nm	350.3	268298
DAR-4M	A rhodamine-based photo-stable nitric oxide (NO) fluorescent indicator with a detection limit of ~10 nM.	~560 nm	~575 nm	430.5	251760
DAR-4M AM	A cell-permeable, photo-stable nitric oxide (NO) fluorescent indicator with a detection limit of ~10 nM. Intracellularly releases DAR-4M (Catalogue No. 251760) by the action of esterases. Reacts with NO, in the presence of O_2, resulting in a triazolo-rhodamine analog (DAR-4M T) that exhibits about 840-fold greater fluorescence quantum efficiency.	~560 nm	~575 nm	630.5	251765
Dihydroethidium	A cell-permeable, chemically-reduced ethidium derivative. Commonly used to analyze respiratory burst in phagocytes.	500 nm	540 nm	315.4	309800
Dihydrorhodamine 123	Cell-permeable fluorogenic probe that is useful for the detection of reactive oxygen species (ROS) such as peroxide and peroxynitrite. Dihydrorhodamine is not fluorescent unless it is converted to rhodamine 123.	NA	NA	346.4	309825
Dimethyl Sulfoxide	ACS Reagent Grade.	NA	NA	78.1	317275
Ethidium Bromide, OmniPur®	Fluorescent stain for illustrating nucleic acids in gels. Facilitates purification of DNA in Cesium Chloride gradient. Molecular biology grade.	545 nm	605 nm	394.32	4310-1GM 4340-5GM
Ethidium Bromide Adsorber	Developed specifically for the safe and simple removal of ethidium bromide (EtBr) from aqueous staining solutions and running buffers used in nucleic acid separation gels.	NA	NA	NA	331569
Ethidium Bromide Solution	Suitable for fluorescent staining of nucleic acids. Molecular biology grade.	545 nm	605 nm	394.32	4410-10ML
FLUO 3/AM	Membrane-permeable version of FLUO 3 (Catalogue No. 343244). Unlike all other Ca^{2+} indicators, FLUO 3/AM is non-fluorescent until it is hydrolyzed in the cell by cellular esterases. It is insensitive to Mg^{2+}.	506 nm	526 nm (after hydrolysis)	1129.9	343242
FSB	A fluorine analog of the amyloidophilic fluorescent probe BSB (Catalogue No. 286895) that crosses the blood-brain barrier and displays low toxicity (LD50 80 mg/kg). Suitable for non-invasive amyloid visualization in living transgenic Tg2576 mice by using 19F and 1H-MRI (Magnetic Resonance Imaging).	NA	NA	420.4	344101

Product Name	Description	Excitation	Emission	Molecular Weight (g/mole)	Catalogue No.
FURA 2/AM	Cell-permeable ester form of FURA 2. Note: 1 set = 10 x 100 µg. PLURONIC® F-127, PROTEIN GRADE® Detergent (Catalogue No. 540025) is a mild, low-toxicity detergent that can aid in the solubilization of FURA 2/AM.	362 nm (low), 335 nm (high)	512 nm (low), 505 nm (high)	1001.9	344905
FURA 2/AM in Solution	Cell-permeable ester form of FURA 2. A 1mM solution in anhydrous DMSO.	362 nm (low), 335 nm (high)	512 nm (low), 505 nm (high)	1001.9	344906
FURA-2 LR/AM	Acetoxymethyl (AM) derivative of FURA-PE3 with spectral properties similar to the parent compound.	368 nm (low), 374 nm (high)	518 nm (low), 524 nm (high)	1258.1	344911
INDO 1/AM	Cell-permeable ester derivative of INDO 1.	349 nm (low), 330 nm (high)	485 nm (low), 398 nm (high)	1009.9	402096
JC-1	A cationic, fluorescent, carbocyanine dye that can be used as a ratiometric indicator of mitochondrial potential in cells, tissues, and isolated mitochondria.	514 nm	529 nm	652.2	420200
MTT	Membrane-permeable yellow dye that is reduced by mitochondrial reductases in living cells to form the dark blue product, MTT-formazan.	NA	NA	414.3	475989
Propidium Iodide	Membrane impermeable DNA intercalator. Has red fluorescence at 488 nm. Useful for flow cytometry for staining apoptotic cells and nuclei.	536 nm	617 nm	668.4	537059
Propidium Iodide Solution	A convenient form of propidium iodide useful for flow cytometry studies.	~ 536 nm	~ 617 nm	668.4	537060
QUIN 2, Tetrapotassium Salt	Binding of calcium to QUIN 2 leads to a major shift in UV absorption and a 20-fold enhancement in fluorescence quantum yield.	354 nm (low), 332 nm (high)	510 nm (low), 505 nm (high)	693.9	551826
RAPIDstain Reagent	An ultrasensitive Coomassie-based reagent for staining polyacrylamide gels.	NA	NA	NA	553215
Rhodamine 123	Membrane-permeable fluorescent dye for selectively staining mitochondria in living cells. Can be used to measure the efflux activity of P-glycoprotein in drug-resistant phenotypes in cancer cells.	~510 nm	~534 nm	380.8	555505
SynaptoGreen Reagent	A lipophilic, non-toxic water-soluble styrylpyridinium green fluoroprobe that is useful for staining active recycling synaptic vesicles. The compound is non-fluorescent in aqueous solution but becomes fluorescent when incorporated into the plasma membrane.	~510 nm	~625 nm	611.6	574798
SynaptoRed Reagent	A lipophilic, water-soluble styrylpyridinium red fluoroprobe that is useful for staining active recycling synaptic vesicles.	~560 nm	~735 nm	607.5	574799
Thioflavin T	A cell-permeable benzothiazole dye that exhibits enhanced fluorescence upon binding to amyloid fibrils. Useful in monitoring stacked β sheet aggregates.	~385 nm-free (~437 nm)	~445 nm-free (~485 nm)	318.9	596200
Thiol Fluorescent Probe IV	A cell-permeable, practically non-fluorescent coumarin cis-acrylate derivative that exhibits a 470-fold increase in fluorescence property.	NA	NA	289.2	595504

Substrates

Product Name	Description	Sizes	Catalogue No.
2-N-Hexadecanoylamino-4-nitrophenylphosphorylcholine	Synthetic chromogenic analog of sphingomyelin. Useful substrate for selectively measuring the activity of acid sphingomyelinase	100 mg	376581
Guanosine 3′,5′-cyclic Monophosphate, 2′-O-(N-Methylanthraniloyl)-, Sodium Salt	A fluorescent, cell-permeable analog of cGMP. Suitable as a substrate for phosphodiesterase studies. Quantum yield: 0.26. Note: 10 µmol = 5.00 mg	10 µmol	370668
4-Methylumbelliferyl-α-D-glucopyranoside	Substrate for fluorogenic assay of α-glucosidase. Has been used in studies of Pompe disease.	100 mg	474426
4-Methylumbelliferyl-β-D-glucuronide	Substrate for fluorogenic assay of β-D-glucuronidase.	100 mg	474427
4-Methylumbelliferylphosphate, Free Acid	Ultrasensitive substrate for fluorometric, phosphorometric, and spectrophotometric assays of phosphatases	1 g	474431
5-Br-4-Cl-3-indolyl-β-D-galactopyranoside	A chromogenic substrate for β-galactosidase. Used to distinguish between recombinant and non-recombinant plasmids carrying β-galactosidase gene (e.g. pUR222)	100 mg, 250 mg, 1 g, 5 g	203782
5-Br-4-Cl-3-indolyl-β-D-glucuronic Acid	Substrate for β-glucuronidase that produces insoluble, intense indigo-blue chromophore (615 nm) after enzymatic hydrolysis. Useful for a variety of applications, including use in bacterial detection systems (e.g. *E. coli*).	100 mg	203783
CENTA™ β-Lactamase Substrate	Chromogenic β-lactamase substrate for both gram-negative and gram-positive bacteria that is comparable to other chromogens (PADAC™ Substrate and nitrocefin). Hydrolysis of the β-lactam ring causes a color change from light yellow (λmax: 340 nm) to chrome yellow (λmax: 405 nm). Relatively unaffected by commonly used microbiological media and human serum. Stable in H_2O and in the presence of cysteine, mercaptoethanol and dimercaptopropanol.	25 mg	219475
Creatine Phosphate, Dipotassium Salt	A high-energy phosphate compound. Transfer of phosphate group from creatine phosphate to ADP and from ATP to creatine form the basis of the creatine-creatine phosphate energy shuttle.	250 mg, 1 g	237911
Creatine Phosphate, Disodium Salt	Creatine phosphate is a high-energy phosphate compound that stores energy from mitochondria whenever sufficient amounts of ATP are present. When ATP levels are low, energy from phosphocreatine is transferred to ADP molecules, which are quickly converted to ATP. It is often a component of ATP-generating systems.	5 g, 25 g	2380
Fluorescein Diacetate	Stains living cells green. Substrate for esterases. Suitable for use in cell viability assays. Removal of the acetate groups by cellular esterases results in fluorescence in cells with intact membranes.	5 g	343209
FTase Substrate, Fluorogenic	A pentapeptide substrate based on the carboxyl terminus of H-Ras with a dansyl group attached to the amino terminus. Serves as a substrate for continuous fluorescent monitoring of protein-farnesyl transferase (FTase) activity (kcat = 0.5 s-1; Km = 1.4 µM) in the presence of farnesyl diphosphate. S-farnesylation of the cysteine moiety results in approximately 13-fold enhancement in fluorescence intensity (monitored at λ = 505 nm) accompanied by a shift in the emission maximum from 565 nm to 515 nm. Reported to be highly selective for FTase and is not recognized by geranylgeranyltransferase I. Useful for screening of potential FTase inhibitors. ε340 = 4,250 M-1 cm-1 in 20 mM Tris-HCl, 10 mM EDTA, pH 7.5.	1 mg	344505
Histone Deacetylase Substrate, Fluorogenic	A fluorogenic substrate for histone deacetylase (HDAC). Excitation maximum: 330 nm; Emission maximum: 395 nm.	1 mg	382155
Methyl-α-D-mannopyranoside	Substrate for α1, 2-Mannosyltransferase (Catalogue No. 444125). Lectin inhibitory sugar.	25 g, 100 g	462711
Methylumbelliferyl-α-D-galactopyranoside	Substrate for the fluorogenic assay of α-D-galactosidase.	50 mg	474422
Methylumbelliferyl-N-Ac-α-D-glucosaminide	Ultrasensitive, fluorogenic substrate for assay of N-acetyl-α-D-glucosaminidase. Can be used to test for Sanfilippo syndrome B.	5 mg	474500
Methylumbelliferyl-N-Ac-β-D-glucosaminide	Ultrasensitive, fluorogenic substrate for assays of N-acetyl-β-D-glucosaminidase	250 mg	474502
Methylumbelliferyl-sulfo-N-Ac-β-D-glucosaminide	Fluorogenic substrate for isoenzyme A of N-acetyl-β-D-glucosaminidase (hexosaminidase A). Can be used for the prenatal diagnosis of Tay-Sachs disease and for the classification of GM2 gangliosidosis genotypes.	25 mg	454428

Substrates

Product Name	Description	Sizes	Catalogue No.
N-Glycan Acceptor Substrate, Dabsylated	A dabsylated tetrapeptide with the N-glycan having the asialo-, agalacto-substructure of biantennary N-linked oligosaccharides found in a wide range of glycoproteins. Useful as a substrate for the non-radioactive assaying by MALDI-mass spectrometry of α1,3- and α1,6-Fuc-transferases, β1,4- and β1,3-Gal-transferases, β1,2-xylosyltransferase, and endo-N-acetylglucosaminidases.	50 nmol	436390
NGB	A chromogenic substrate for direct and continuous assay of arginase (Km = 1.6 mM, kcat = 0.09 min-1) with a detection limit of ~1 µg/mL. Arginase hydrolyzes NGB to yield urea plus the chromophore m-nitroaniline (λ = 372 nm, ε = 1,280 M-1cm-1). Although the kcat value for NGB hydrolysis is much lower than arginine, the Km value (1.6 mM) is nearly identical to arginine (Km = 1.4 mM)	10 mg	481490
Nitrocefin	A chromogenic β-lactamase substrate that undergoes distinctive color change from yellow (λmax = 390 nm at pH 7.0) to red (λmax = 486 nm at pH 7.0) as the amide bond in the β-lactam ring is hydrolyzed by β-lactamase. Nitrocefin is sensitive to hydrolysis by all known lactamases produced by Gram-positive and Gram-negative bacteria. Also useful for the detection of β-lactamase patterns from bacterial cell extracts by isoelectric focusing. Has been used in competitive inhibition studies in developmental work on β-lactamase-resistant antibiotics.	5 mg	484400
o-Nitrophenyl-β-D-galactopyranoside	A β-galactosidase substrate for colorimetric and EIA applications; counterpart of widely used pNPP/alkaline phosphatase substrate. o-Nitrophenol is produced as the end product and is monitored at 405 nm.	1 g, 5 g	48712
p-Nitroblue Tetrazolium Chloride	NADPH-diaphorase substrate that competitively inhibits nitric oxide synthase (IC_{50} = 3-4 µM). A well-known scavenger of superoxide anions. Useful as a substrate for alkaline phosphatase, often in conjunction with BCIP (Catalogue No. 203788).	1 g	484235
p-Nitrophenyl-α-D-glucopyranoside	Chromogenic substrate for α-glucosidase.	1 g, 5 g	487506
p-Nitrophenyl-α-D-maltopentaoside	Suitable for determination of α-amylase activity.	10 mg	487526
p-Nitrophenyl-α-D-maltoside	Useful substrate for assay of salivary α-amylase activity.	50 mg	487542
p-Nitrophenyl-β-D-glucopyranoside	Chromogenic substrate for β-glucosidase.	1 g	487507
p-Nitrophenyl-β-D-glucuronide	Chromogenic substrate for β-glucuronidase (Km = ~0.22 mM)	1 g	487500
p-Nitrophenyl-β-D-xylopyranoside	Substrate for the colorimetric assay of β-D-xylosidase. Also shown to disrupt proteoglycan biosynthesis *in vivo*.	1 g	487870
p-Nitrophenyl-N-acetyl-β-D-glucosaminide	Useful substrate for rapid colorimetric assay of N-acetyl-β-glucosaminidase activity in human urine.	1 g	487052
Phenolphthalein Glucuronide, Sodium Salt	Substrate for β-glucuronidase.	50 mg	516735
PSA Substrate, Fluorogenic	An excellent fluorogenic substrate for assaying the proteolytic activity of prostate-specific antigen (PSA).	1 mg	539582
S-Butyrylthiocholine Iodide	Chromogenic substrate for cholinesterases. Cleavage is measured colorimetrically at 405 nm.	25 g	203989
SNAPtide BoTox A Substrate, Fluor.	A synthetic peptide, fluorescence resonance energy transfer (FRET) substrate containing the N-terminally-linked fluorophore o-aminobenzoic acid (Abz) and the acceptor chromophore 2, 4-dinitrophenol (DNP) linked to the C-terminal cysteine. The substrate is based on the native botulinum toxin type A cleavage site found in the synaptosome-associated protein SNAP-25. Cleavage of substrate by botulinum toxin releases the fluorescence is restored.	200 nmol	567333
T Antigen, *p*-Nitrophenyl-	Useful as a substrate for α2,3-(O)-Sialyltransferase (Catalogue No. 566227).	5 mg	575303

Plasma Proteins

Product Name	Description	Molecular Weight kDa	Purity	Sizes	Catalogue No.
Advanced Glycation Endproduct-BSA	Prepared by reacting BSA with glycoaldehyde under sterile conditions.	NA	≥95% by SDS-PAGE	10 mg	121800
Albumin, Bovine Serum, 10% Aqueous Solution, Nuclease-Free	Useful for applications in which acetylated BSA is not desirable, such as in antibody dilution, DNA footprinting, and gel shift assay, ELISA, enzyme assay, enzyme stabilization, immunoblotting, immunofluorescence, PCR, restriction enzyme reactions, RIA, probe-based diagnostics, radioactive quenching, and receptor binding studies.	66	NA	25 mL	126615
Albumin, Bovine Serum, 30% Aqueous Solution, Stabilizer-Free	Highly purified preparation suitable for a variety of *in vitro* assays.	66	≥96% by Cellulose acetate electrophoresis	50 mL	126625
Albumin, Bovine Serum, 30% Sterile-Filtered Aqueous Solution, Preservative-Free	Useful as a stabilizing agent and carrier protein.	66	≥96% by Agarose Electrophoresis	50 mL	126626
Albumin, Bovine Serum, Cohn Fraction, 30% Aqueous Solution	Purified by cold ethanol extraction (Cohn extraction) and by heat treatment to remove lipids and fatty acids.	66	≥95% by agarose electrophoresis	50 mL	126621
Albumin, Bovine Serum, Fraction V, Crystalline	Prepared by cold ethanol extraction (Cohn method) followed by crystallization.	66	≥99% by cellulose acetate electrophoresis	1 g, 5 g, 25 g	12657
Albumin, Bovine Serum, Fraction V, Fatty Acid-Free	Useful for immunodiagnostic assays. pH 6.8-7.4.	66	≥98% by SDS-PAGE	10 g, 100 g	126575
Albumin, Bovine Serum, Fraction V, Fatty Acid-Free, Nuclease- and Protease-Free	Designed for use in serological testing, RIA, and hormone response studies.	66	≥98% by SDS-PAGE	5 g, 10 g, 100 g	126609
Albumin, Bovine Serum, Fraction V, Fatty Acid-Poor, Endotoxin-Free	Designed for use in cell culture systems and animal perfusion studies.	66	≥95% by SDS-PAGE	10 g, 100 g	126579
Albumin, Bovine Serum, Fraction V, Low Heavy Metals	Prepared by heat shock treatment procedure.	66	≥98% by SDS-PAGE	25 g, 100 g, 250 g, 500 g, 1 kg	12659
Albumin, Bovine Serum, Fraction V, Modified Cohn, pH 5.2	Native fatty acid profile reflects endogenous lipids. Has a pH of 5.2 in 150 mM NaCl (1%).	66	≥96% by SDS-PAGE	100 g, 1 kg	12660
Albumin, Bovine Serum, Fraction V, RIA and ELISA Grade	Prepared under non-denaturing conditions by a modification of Cohn procedure and caprylic acid heat-shock treatment. Has a pH of 6.8-7.4 at 10% in 150 mM NaCl.	66	≥98% by SDS-PAGE	10 g, 25 g, 100 g	126593
Albumin, Chicken Egg, 5X Crystalline	Used as a carrier and stabilizer protein.	45	≥98% by SDS-PAGE	1 g, 5 g	32467
Albumin, Human Serum, 30% Aqueous Solution	Prepared from serum that has been shown by certified tests to be negative for HBsAg and for antibodies to HIV and HCV.	66	≥95% by SDS-PAGE	50 mL	12667
Albumin, Human Serum, Fraction V	Prepared under non-denaturing conditions by a modification of the Cohn procedure.	66	≥95% by SDS-PAGE	10 g, 100 g	12668

Plasma Proteins

Product Name	Description	Molecular Weight kDa	Purity	Sizes	Catalogue No.
Albumin, Human Serum, Fraction V, High Purity	Highly purified preparation.	66	≥95% by cellulose acetate electrophoresis	1 g, 5 g	126658
Albumin, Human Serum, Fraction V, Low Heavy Metals	Prepared under non-denaturing conditions by a modification of the Cohn procedure.	66	≥95% by SDS-PAGE	1 g, 10 g, 100 g	12666
Albumin, Human Serum, Non-denatured	High purity albumin prepared under non-denaturing conditions.	66	≥95% by SDS-PAGE	1 g	126654
Albumin, Mouse	Plasma protein that is useful as a stabilizing agent and a carrier protein.	69	≥98% by agarose electrophoresis	25 g	126674
Albumin, Rat	Plasma protein that is useful as a stabilizing agent and a carrier protein.	69	≥98% by agarose electrophoresis	100 mg	126722
Angiotensinogen, Human Plasma	Native angiotensinogen from human plasma. An α_2 globulin present in plasma at about 7 mg/ 100 mL. Can be cleaved by renin to generate angiotensin I.	52	≥95% by SDS-PAGE	100 µg	176870
Apolipoprotein A-I, Human Plasma, High-Density Lipoprotein	Native apolipoprotein A-I from human plasma. Functions as a cofactor for lecithin-cholesterol acyltransferase (LCAT). Plays an important role in HDL metabolism.	28	≥95% by SDS-PAGE	500 µg	178452
Apolipoprotein A-II, Human Plasma, High-Density Lipoprotein	Native apolipoprotein A-II from human plasma. Shown to bind to phospholipids during lipoprotein metabolism. Displaces lecithin-cholesterol acyltransferase bound to lipoprotein. Influences HDL functional states and contributes to arteriosclerosis.	17	≥95% by SDS-PAGE	500 µg	178455
Apolipoprotein B, Human Plasma, Low-Density Lipoprotein	Native apolipoprotein B from human plasma. Produced by the liver, and is a dominant protein constituent of LDL that is also present in VLDL. Ligand for LDL receptor, directing clearance of LDL from plasma to the liver. Functions as a cofactor in enzymatic reactions. Mean serum concentration: 950 µg/mL.	550	≥95% by SDS-PAGE	500 µg	178456
Apolipoprotein C-I, Human Plasma, Very Low-Density Lipoprotein	Native apolipoprotein C-I from human plasma. A major component of VLDL that partially activates lecithin-cholesterol acyltransferase activity and inhibits lipoprotein lipase activity.	6.6	≥95% by SDS-PAGE	100 µg	178459
Apolipoprotein C-II, Human Plasma, Very Low-Density Lipoprotein	Native apolipoprotein C-II from human plasma. Found primarily in VLDL and chylomicrons. Functions as a cofactor for lipoprotein lipase.	8.8	≥95% by SDS-PAGE	50 µg	178462
Apolipoprotein C-III, Human Plasma, Very Low-Density Lipoprotein	Native apolipoprotein C-III from human plasma. Major protein of VLDL and chylomicrons. Involved in the uptake of triglycerides by cells. May inhibit the activation of lipoprotein lipase by Apo C-II. Present in normal plasma at 80-150 µg/mL.	8.8	≥95% by SDS-PAGE	100 µg	178461
Apolipoprotein E, Human Plasma, Very Low-Density Lipoprotein	Native apolipoprotein E from human plasma. A component of VLDL and a subclass of HDL. Serves as a ligand for LDL receptors, where it participates in the transport and redistribution of cholesterol and other lipids.	34	≥95% by SDS-PAGE	50 µg	178468
Bovine γ-Globulin, Serum	Native γ-globulin from bovine serum.	158	≥97% by electrophoresis	5 g, 25 g, 100 g	345876

Plasma Proteins

Product Name	Description	Molecular Weight kDa	Purity	Sizes	Catalogue No.
Ceruloplasmin, Human Plasma	Native ceruloplasmin from human plasma. Serum copper transport and iron-oxidizing protein. Plays an important role in antioxidant protection against organic and inorganic oxygen radicals generated by iron and ascorbate.	134	≥95% by SDS-PAGE	1 mg	239799
Chicken γ-Globulin, Serum	Native γ-globulin from chicken serum.	125	≥85% by electrophoresis	100 mg	345877
C-Reactive Protein, Human Ascites	Native C-reactive protein from human ascites. CRP is a major acute-phase plasma protein that is dramatically elevated in patients with acute-phase conditions. Composed of five identical noncovalently linked subunits. Important in diagnosis of rheumatoid arthritis. Suitable for immunological studies.	21	Single band by SDS-PAGE	1 mg	236600
C-Reactive Protein, Human Serum, High Purity	Native C-reactive protein highly purified from human serum. Important in diagnosis of rheumatoid arthritis. Suitable for immunological studies. Intended for use as a calibration standard for assays of CRP, for structural and functional studies, and as an antigen for production of antisera.	21	≥99% by SDS-PAGE	100 µg	236603
C-Reactive Protein, Human, Recombinant, *E. coli*	Recombinant, human C-reactive protein expressed in *E. coli*. CRP is a major acute-phase plasma protein that is dramatically elevated in patients with acute-phase conditions. Suitable for immunological studies.	21	Single band by SDS-PAGE	1 mg	236608
Ferritin, Apo-, Equine Spleen	Native apo-ferritin from equine spleen. Protein shell of ferritin molecule lacking iron. Large amounts are present in pancreatic β-cells where it acts as an antioxidant.	460	≥90% by SDS-PAGE	100 mg	178440
Ferritin, H-Chain, Human, Recombinant, *E. coli*	Recombinant, human ferritin H-chain expressed in *E. coli*. Contains the metal binding site of ferritin that confers ferroxidase activity to the protein.	507	≥95% by SDS-PAGE	25 µg	341490
Ferritin, Human Liver	Native ferritin from human liver. Sterile-filtered. A major iron storage protein. Suitable for use in immunoassays, as an immunogen, and in enzyme/radiolabeling.	450	≥95% by SDS-PAGE	1 mg	341482
Fetuin, Fetal Bovine Serum	Native fetuin from fetal bovine serum. An α-globulin that inhibits trypsin activity and promotes cell attachment, growth, and differentiation in many different culture systems. Also contains both N- and O-glycosidically-linked carbohydrates. Functions as an opsonin for cationic-deactivating molecules in macrophages.	48.7	≥70% by electrophoresis	1 g	341506
Gc-Globulin, Mixed Type, Human Plasma	Native Gc-globulin from human plasma. Single polypeptide chain present in plasma at about 20-55 mg/dl. Functions in binding and transport of vitamin D. Binds to cell surface at the DBP binding site in a nonsaturable manner.	52	≥95% by SDS-PAGE	1 mg	345802
Haptoglobin, Mixed Type, Human Plasma	Native haptoglobin from human plasma. A hemoglobin-binding acute-phase protein that promotes cholesterol crystallization and is found in human plasma at 82 to 236 mg per deciliter. It is a tetramer composed of α- and β-polypeptide chains.	86	≥95% by SDS-PAGE	1 mg	372022

Plasma Proteins

Product Name	Description	Molecular Weight kDa	Purity	Sizes	Catalogue No.
Hemoglobin, Bovine Erythrocytes	Native hemoglobin from bovine erythrocytes. A major oxygen-transporting component of red blood cells that is also nitric oxide scavenger. Blocks carbachol-stimulated cGMP production. This preparation contains primarily ferric-hemoglobin and must be reduced to the ferrous form to bind molecular oxygen.	64.5	≥95% by SDS-PAGE	5 g	3745
Hemoglobin, Bovine Erythrocytes	Native hemoglobin from bovine erythrocytes. Excellent substrate for proteases, such as papain, pepsin, and trypsin.	64.5	NA	10 g	374834
Human IgA1, Plasma	Native IgA 1 purified from human plasma.	160	≥95% by SDS-PAGE	500 µg	400105
Human IgD, Myeloma Plasma	Native IgD from human myeloma plasma.	185	≥95% by SDS-PAGE	100 µg	401164
Human IgG, Fab Fragment, Plasma	Native IgG Fab fragment derived from normal human plasma IgG.	55	≥95% by SDS-PAGE	2 mg	401116
Human γ-Globulin, Serum	Native γ-globulin from human serum.	155	≥90% by electrophoresis	1 g, 5 g	345886
Lipoproteins, High Density, Human Plasma	Native high density lipoproteins from human plasma. Cholesterol-carrier lipoprotein that acts as scavenger of tissue cholesterol. Important in cholesterol efflux from tissues. Involved in return of cholesterol from the periphery to the liver for removal as bile acids. Composition: 55-45% lipid, 45-55% protein.	NA	≥95% of total lipoprotein content by electrophoresis	10 mg	437641
Lipoproteins, Low Density, Human Plasma	Native low density lipoproteins from human plasma. Cholesterol-carrier lipoprotein responsible for delivery of lipids (cholesterol) from liver to tissues. Composition: 78-81% lipid; 19-22% protein.	NA	≥95% of total lipoprotein content by electrophoresis	10 mg	437644
Lipoproteins, Very Low Density, Human Plasma	Native very low density lipoproteins from human plasma. VLDL transports liver-synthesized triglycerides and cholesterol. May counteract the inhibitory effect of glucocorticoids on arachidonic acid release and prostaglandin I2 formation in vascular smooth muscle cells. Composition: 88-95% lipid; 5-12% protein.	NA	≥95% of total lipoprotein content by electrophoresis	5 mg	437647
Mouse IgG_{2a}, Myeloma	Native IgG_{2a} from mouse myeloma.	150	Purity by ELISA	1 mg	401123
Normal Donkey Serum, Sterile	Normal carrier sera, produced from pooled lots of sera from healthy, non-immunized donkeys. Suitable for use in radioimmunoassay.	NA	NA	5 mL	566460
Normal Goat Serum, Lyophilized Solid	Normal carrier sera, produced from pooled lots of sera from healthy, non-immunized goats. Suitable for use in radioimmunoassay.	NA	NA	10 mL	566380
Normal Guinea Pig Serum, Lyophilized Solid	Normal carrier sera, produced from pooled lots of sera from healthy, non-immunized guinea pig. Suitable for use in radioimmunoassay.	NA	NA	5 mL	566400
Normal Rabbit Serum, Lyophilized Solid	Normal carrier sera, produced from pooled lots of sera from healthy, non-immunized rabbits. Suitable for use in radioimmunoassay.	NA	NA	5 mL	869019
BSA,Fraction V, OmniPur®	Cold Alcohol Isolation	66	≥99% by electrophoresis	5 g	2905-5GM
OmniPur® BSA,Fraction V heat shock isolation	Isolated from domestically sourced bovine serum in a closed-loop process.	66	≥98 NA%	25 g	2910

Plasma Proteins

Product Name	Description	Molecular Weight kDa	Purity	Sizes	Catalogue No.
OmniPur® BSA,Fraction V heat shock isolation	Isolated from domestically sourced bovine serum in a closed-loop process.	66	≥98%	100 g	2930
OmniPur® BSA,Fraction V heat shock isolation	Isolated from domestically sourced bovine serum in a closed-loop process.	66	≥98%	500 g	2960
OmniPur® BSA,Fraction V heat shock isolation	Isolated from domestically sourced bovine serum in a closed-loop process.	66	≥98%	1 kg	2980
Plasminogen, EACA- and Lysine-Free, Human Plasma	Native plasminogen (EACA- and lysine-free) from human plasma. Plasminogen is a single chain glycoprotein found in normal plasma at ~12 mg per deciliter, and is the inactive precursor of the serine protease plasmin.	90	NA	50 u	528178
Plasminogen, Glu-Type, Human Plasma	Native Glu-type plasminogen from human plasma. Plasminogen is a single chain glycoprotein found in normal plasma at ~12 mg per deciliter, and is the inactive precursor of the serine protease plasmin.	90	Single band by SDS-PAGE	1 mg	528180
Plasminogen, Human Plasma	Native plasminogen from human plasma. Plasminogen is a single chain glycoprotein found in normal plasma at ~12 mg per deciliter, and is the inactive precursor of the serine protease plasmin.	90	≥95% by SDS-PAGE	120 u	528175
Plasminogen, Lys-Type, Human Plasma	Native Lys-type plasminogen from human plasma prepared from homogeneous Glu-plasminogen by activation with plasmin. Activation results in the release of a 76 residue peptide (Glu1 - Lys76) fragment. Lys77-plasminogen can be readily converted to Lys77-plasmin by any of the common plasminogen activators.	83	Homogeneous by SDS-PAGE	1 mg	528185
Platelet Factor 4, Human Platelets	Native platelet factor 4 from human platelets. A heparin-binding protein released from α-granules of activated platelets. Binds to the newly formed blood vessels and reduces tumor neovascularization.	78-90	≥95% by SDS-PAGE	100 µg	521726
Platelet Membrane Glycoproteins, IIbIIIa, Human Platelets	Native, platelet membrane glycoprotein IIbIIIaA from human platelets. A platelet integrin that binds to fibrinogen via the recognition sequence Arg-Gly-Asp-Ser. Acts as a receptor for such adhesive ligands as fibrinogen, fibronectin and von Willebrand factor during platelet stimulation. Glycoproteins IIb and IIIa constitute the fibrinogen receptor and are required for platelet aggregation. Glycoprotein IIb consists of two disulfide-linked subunits, GPIIbα and GPIIbβ, whereas GPIIIa has only one polypeptide chain.	NA	NA	1 mg	528240
Prealbumin, Human Plasma	Native prealbumin from human plasma. Plasma protein with minimal carbohydrate. Found in plasma at about 30 mg/100 mL. Functions in the transport of retinol-binding protein, thyroxine, and vitamin A.	55	≥95% by SDS-PAGE	1 mg	529577
Prekallikrein, Human Plasma	Native prekallikrein from human plasma. Single-chain glycoprotein that participates in the early phase of blood coagulation, kinin formation, and fibrinolysis.	86	≥95% by SDS-PAGE	1 mg	529583
Prionex® Reagent	A unique alternative to bovine serum albumin (BSA). Prionex® Reagent is a polypeptide fraction of highly purified dermal collagen of porcine origin that is free from cartilage, bone and plasma components and that can be considered as an extremely pure form of gelatin type A. Used as an inert protein stabilizer in many applications. Also useful as a blocking agent and as a protective additive in cell culture.	20	NA	100 mL	529600

OmniPur® Grade - DNase, RNase, and protease tested

Plasma Proteins

Product Name	Description	Molecular Weight kDa	Purity	Sizes	Catalogue No.
Protein C, Activated, Human Plasma	Native, activated protein C from human plasma. Glycoprotein composed of a heavy chain and a light chain held together by a disulfide bond. Its natural substrates are activated forms of clotting Factors V and VIII.	61	NA	10 µg	539218
Protein C, Human Plasma	Native protein C from human plasma. Sold as a zymogen. Markedly prolongs kaolin-cephalin clotting time of normal plasma. Activated by α-thrombin to serine protease-activated protein C. In its active form, it is a potent anticoagulant that acts through selective inactivation of Factors Va and VIIIa.	62	≥95% by SDS-PAGE	50 µg , 100 µg	539215
Protein S, Human Plasma	Native protein S from human plasma. Single-chain glycoprotein that functions as a cofactor for the anticoagulant activity of activated protein C.	69	≥95% by SDS-PAGE	100 µg	539406
Prothrombin, Human Plasma	Native prothrombin from human plasma. Vitamin K-dependent glycoprotein synthesized in the liver. Conversion to thrombin is a key step in the blood coagulation pathway and catalyzes the coagulation of fibrinogen. Clinically, cases of selective deficiency are rare, although, in cases of liver cirrhosis, prothrombin is decreased.	72	≥95% by SDS-PAGE	1 mg, 2 mg	539515
Rabbit IgG, Plasma	Native IgG from rabbit plasma. IgG is a glycoprotein found in serum and tissue fluids. For use as a negative control in parallel with specific rabbit IgG-type primary antibodies.	NA	≥98% by SDS-PAGE	50 mg	401590
Rabbit γ-Globulin, Serum	Native γ-globulin from rabbit serum.	NA	≥95% by electrophoresis	1 g, 5 g	345991
Transferrin Apo-, Human Plasma, Low Endotoxin Level	Native apotransferrin from human plasma. Transferrin is a serum protein involved in iron binding and transport. Substantially iron-free. Cell culture-tested.	80	≥95% by SDS-PAGE	10 mg	616419
Transferrin, Apo-, Human Plasma	Native apo-transferrin from human plasma. Transferrin is a serum protein involved in iron binding and transport. Substantially iron-free.	80	≥95% by SDS-PAGE	100 mg	616395
Transferrin, Apo-, Low Endotoxin Grade, Human Plasma	Native apotransferrin from human plasma. Transferrin is a serum protein involved in iron binding and transport. Substantially iron-free. Cell culture-tested.	80	≥95% by SDS-PAGE	100 mg, 1 g	178481
Transferrin, Holo, Bovine Plasma	Native holo-transferrin from bovine plasma. Transferrin is a serum protein involved in iron binding and transport.	77	≥95% by FPLC	100 mg	616420
Transferrin, Holo, Human Plasma	Native holotransferrin from human plasma. Transferrin is a serum protein involved in iron binding and transport.	80	≥95% by SDS-PAGE	100 mg, 500 mg	616397
Transferrin, Holo, Human Plasma, Low Endotoxin	Native holotransferrin from human plasma. Transferrin is a serum protein involved in iron binding and transport.	80	≥95% by SDS-PAGE	100 mg	616424