ZÁKLADNÍ ROZTOKY POUŽÍVANÉ V MOLEKULÁRNÍ BIOLOGII

TABLE B.7 *(continued)*

Solution	Method of preparation	Comments
0.1 M Adenosine triphosphate (ATP)	Dissolve 60 mg of ATP in 0.8 ml of H_2O. Adjust the pH to 7.0 with 0.1 N NaOH. Adjust the volume to 1 ml with distilled H_2O. Dispense the solution into small aliquots and store at −70°C.	
10 M Ammonium acetate	Dissolve 770 g of ammonium acetate in 800 ml of H_2O. Adjust the volume to 1 liter with H_2O. Sterilize by filtration.	
10% Ammonium persulfate	To 1 g of ammonium persulfate, add H_2O to 10 ml. The solution may be stored for several weeks at 4°C.	
BCIP	Dissolve 0.5 g of 5-bromo-4-chloro-3-indolyl phosphate disodium salt, which is available from several manufacturers, in 10 ml of 100% dimethylformamide. Store at 4°C.	Initially, the BCIP may not dissolve completely (especially if the dimethylformamide is not very fresh). If this occurs, vortex the mixture to suspend the BCIP and then withdraw the desired amount using a pipette tip cut off to make a large bore. The BCIP will dissolve fully in the next step of the protocol.
2× BES-buffered saline	Dissolve 1.07 g of BES (*N,N*-bis[2-hydroxyethyl]-2-aminoethanesulfonic acid), 1.6 g of NaCl, and 0.027 g of Na_2HPO_4 in a total volume of 90 ml of distilled H_2O. Adjust the pH of the solution to 6.96 with HCl at room temperature, and then adjust the volume to 100 ml with distilled H_2O. Sterilize the solution by passage through a 0.22-micron filter, and store in aliquots at −20°C.	
1 M $CaCl_2$	Dissolve 54 g of $CaCl_2 \cdot 6H_2O$ in 200 ml of pure H_2O (Milli-Q or equivalent). Sterilize the solution by passage through a 0.22-micron filter. Store in 1-ml aliquots at −20°C.	When preparing competent cells, thaw an aliquot and dilute it to 100 ml with pure H_2O. Sterilize the solution by filtration through a Nalgene filter (0.45-micron pore size), and then chill it to 0°C.
2.5 M $CaCl_2$	Dissolve 13.5 g of $CaCl_2 \cdot 6H_2O$ in 20 ml of distilled H_2O. Sterilize the solution by passage through a 0.22-micron filter. Store in 1-ml aliquots at −20°C.	
Deoxyribonucleoside triphosphates (dNTPs)	Dissolve each dNTP in H_2O at an approximate concentration of 100 mM. Using 0.05 M Tris base and a micropipette, adjust the pH of each of the solutions to 7.0 (use pH paper to check the pH). Dilute an aliquot of the neutralized dNTP appropriately, and read the optical density at the wavelengths given in the table	

below. Calculate the actual concentration of each dNTP. Dilute the solutions with H_2O to a final concentration of 50 mM dNTP. Store each separately at −70°C in small aliquots.

Base	Wavelength (nm)	Extinction Coefficient (ϵ) (M^{-1} cm^{-1})
A	259	1.54×10^4
G	253	1.37×10^4
C	271	9.10×10^3
T	260	7.40×10^3

For a cuvette with a path length of 1 cm, absorbance = ϵM.

100 mM stock solutions of each dNTP are commercially available (Pharmacia) if you do not want to prepare your own.

1 M Dithiothreitol (DTT)

Dissolve 3.09 g of DTT in 20 ml of 0.01 M sodium acetate (pH 5.2). Sterilize by filtration. Dispense into 1-ml aliquots and store at −20°C.

Do not autoclave DTT or solutions containing DDT.

0.5 M EDTA (pH 8.0)

Add 186.1 g of disodium ethylenediaminetetraacetate · $2H_2O$ to 800 ml of H_2O. Stir vigorously on a magnetic stirrer. Adjust the pH to 8.0 with NaOH (~20 g of NaOH pellets). Dispense into aliquots and sterilize by autoclaving.

The disodium salt of EDTA will not go into solution until the pH of the solution is adjusted to approximately 8.0 by the addition of NaOH.

Ethidium bromide (10 mg/ml)

Add 1 g of ethidium bromide to 100 ml of H_2O. Stir on a magnetic stirrer for several hours to ensure that the dye has dissolved. Wrap the container in aluminum foil or transfer the solution to a dark bottle and store at room temperature.

Caution: Ethidium bromide is a powerful mutagen and is moderately toxic. Gloves should be worn when working with solutions that contain this dye, and a mask should be worn when weighing it out. After use, these solutions should be decontaminated by one of the methods described in Appendix E.

2× HEPES-buffered saline

Dissolve 1.6 g of NaCl, 0.074 g of KCl, 0.027 g of $Na_2HPO_4 \cdot 2H_2O$, 0.2 g of dextrose, and 1 g of HEPES in a total volume of 90 ml of distilled H_2O. Adjust the pH to 7.05 with 0.5 N NaOH, and then adjust the volume to 100 ml with distilled H_2O. Sterilize the solution by passage through a 0.22-micron filter. Store in 5-ml aliquots at −20°C.

IPTG

Isopropylthio-β-D-galactoside (m.w. = 238.3). Make a solution of IPTG by dissolving 2 g of IPTG in 8 ml of distilled H_2O. Adjust the volume of the solution to 10 ml with distilled H_2O and sterilize by filtration through a 0.22-micron disposable filter. Dispense the solution into 1-ml aliquots and store them at −20°C.

TABLE B.7 *(continued)*

Solution	Method of preparation	Comments
1 M Magnesium acetate	Dissolve 214.46 g of magnesium acetate · $4H_2O$ in 800 ml of H_2O. Adjust the volume to 1 liter with H_2O. Sterilize by filtration.	
1 M $MgCl_2$	Dissolve 203.3 g of $MgCl_3 \cdot 6H_2O$ in 800 ml of H_2O. Adjust the volume to 1 liter with H_2O. Dispense into aliquots and sterilize by autoclaving.	$MgCl_2$ is extremely hygroscopic. Buy small bottles (e.g., 100 g) and do not store opened bottles for long periods of time.
β-Mercaptoethanol (BME)	Usually obtained as a 14.4 M solution. Store in a dark bottle at 4°C.	Do not autoclave BME or solutions containing BME.
NBT	Dissolve 0.5 g of nitro blue tetrazolium chloride, which is available from several manufacturers, in 10 ml of 70% dimethylformamide. Store at 4°C.	
Phenol:chloroform	Mix equal amounts of phenol and chloroform. Equilibrate the mixture by extracting several times with 0.1 M Tris · Cl (pH 7.6). Store the equilibrated mixture under an equal volume of 0.01 M Tris · Cl (pH 7.6) at 4°C in dark glass bottles.	**Caution:** Phenol is highly corrosive and can cause severe burns. Wear gloves, protective clothing, and safety glasses when handling phenol. All manipulations should be carried out in a chemical hood. Any areas of skin that come into contact with phenol should be rinsed with a large volume of water and washed with soap and water. Do *not* use ethanol.
10 mM Phenylmethylsulfonyl fluoride (PMSF)	Dissolve PMSF in isopropanol at a concentration of 1.74 mg/ml (10 mM). Divide the solution into aliquots and store at −20°C. If necessary, stock solutions can be prepared in concentrations as high as 17.4 mg/ml (100 mM).	**Caution:** PMSF is extremely destructive to the mucous membranes of the respiratory tract, the eyes, and skin. It may be fatal if inhaled, swallowed, or absorbed through the skin. In case of contact, immediately flush eyes or skin with copious amounts of water. Discard contaminated clothing. PMSF is inactivated in aqueous solutions. The rate of inactivation increases with pH and is faster at 25°C than at 4°C. The half-life of a 20 μM aqueous solution of PMSF is about 35 minutes at pH 8.0 (James 1978). This means that aqueous solutions of PMSF can be safely discarded after they have been rendered alkaline (pH > 8.6) and stored for several hours at room temperature.
Phosphate-buffered saline (PBS)	Dissolve 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4, and 0.24 g of KH_2PO_4 in 800 ml of distilled H_2O. Adjust the pH to 7.4 with HCl. Add H_2O to 1 liter. Dispense the solution into aliquots and sterilize them by autoclaving for 20 minutes at 15 lb/sq. in. on liquid cycle. Store at room temperature.	

1 M Potassium acetate (pH 7.5)	Dissolve 9.82 g of potassium acetate in 90 ml of pure H_2O (Milli-Q or equivalent). Adjust the pH to 7.5 with 2 M acetic acid. Add pure H_2O to 100 ml. Divide the solution into aliquots and store them at −20°C.	
Potassium acetate (for alkaline lysis)	To 60 ml of 5 M potassium acetate, add 11.5 ml of glacial acetic acid and 28.5 ml of H_2O. The resulting solution is 3 M with respect to potassium and 5 M with respect to acetate.	
3 M Sodium acetate (pH 5.2 and pH 7.0)	Dissolve 408.1 g of sodium acetate · $3H_2O$ in 800 ml of H_2O. Adjust the pH to 5.2 with glacial acetic acid or adjust the pH to 7.0 with dilute acetic acid. Adjust the volume to 1 liter with H_2O. Dispense into aliquots and sterilize by autoclaving.	
5 M NaCl	Dissolve 292.2 g of NaCl in 800 ml of H_2O. Adjust the volume to 1 liter with H_2O. Dispense into aliquots and sterilize by autoclaving.	
10% Sodium dodecyl sulfate (SDS) (also called sodium lauryl sulfate)	Dissolve 100 g of electrophoresis-grade SDS in 900 ml of H_2O. Heat to 68°C to assist dissolution. Adjust the pH to 7.2 by adding a few drops of concentrated HCl. Adjust the volume to 1 liter with H_2O. Dispense into aliquots.	Wear a mask when weighing SDS and wipe down the weighing area and balance after use because the fine crystals of SDS disperse easily. There is no need to sterilize 10% SDS.
20× SSC	Dissolve 175.3 g of NaCl and 88.2 g of sodium citrate in 800 ml of H_2O. Adjust the pH to 7.0 with a few drops of a 10 N solution of NaOH. Adjust the volume to 1 liter with H_2O. Dispense into aliquots. Sterilize by autoclaving.	
20× SSPE	Dissolve 175.3 g of NaCl, 27.6 g of $NaH_2PO_4 \cdot H_2O$ and 7.4 g of EDTA in 800 ml of H_2O. Adjust the pH to 7.4 with NaOH (~6.5 ml of a 10 N solution). Adjust the volume to 1 liter with H_2O. Dispense into aliquots. Sterilize by autoclaving.	
Trichloroacetic acid (TCA) 100% solution	To a bottle containing 500 g of TCA, add 227 ml of H_2O. The resulting solution will contain 100% (w/v) TCA.	
1 M Tris	Dissolve 121.1 g of Tris base in 800 ml of H_2O. Adjust the pH to the desired value by adding concentrated HCl. **pH** **HCl** 7.4 70 ml 7.6 60 ml 8.0 42 ml	If the 1 M solution has a yellow color, discard it and obtain better quality Tris. Although many types of electrodes do not accurately measure the pH of Tris solutions, suitable electrodes can be obtained from most manufacturers. The pH of Tris solutions is temperature-dependent and decreases approximately 0.03 pH

TABLE B.7 *(continued)*

Solution	Method of preparation	Comments
1 M Tris *(continued)*	Allow the solution to cool to room temperature before making final adjustments to the pH. Adjust the volume of the solution to 1 liter with H_2O. Dispense into aliquots and sterilize by autoclaving.	units for each 1°C increase in temperature. For example, a 0.05 M solution has pH values of 9.5, 8.9, and 8.6 at 5°C, 25°C, and 37°C, respectively.
Tris-buffered saline (TBS) (25 mM Tris)	Dissolve 8 g of NaCl, 0.2 g of KCl, and 3 g of Tris base in 800 ml of distilled H_2O. Add 0.015 g of phenol red and adjust the pH to 7.4 with HCl. Add distilled H_2O to 1 liter. Dispense the solution into aliquots and sterilize them by autoclaving for 20 minutes at 15 lb/sq. in. on liquid cycle. Store at room temperature.	
X-gal	5-Bromo-4-chloro-3-indolyl-β-D-galactoside. Make a stock solution by dissolving X-gal in dimethylformamide to make a 20 mg/ml solution. Use a glass or polypropylene tube. The tube containing the solution should be wrapped in aluminum foil to prevent damage by light and should be stored at −20°C. It is not necessary to sterilize X-gal solutions by filtration.	

TABLE B.9 Proteolytic Enzymes

	Stock solution	Storage temperature	Concentration in reaction	Reaction buffer	Temperature	Pretreatment
Pronase[a]	20 mg/ml in H_2O	−20°C	1 mg/ml	0.01 M Tris (pH 7.8) 0.01 M EDTA 0.5% SDS	37°C	self-digestion[b]
Proteinase K[c]	20 mg/ml in H_2O	−20°C	50 μg/ml	0.01 M Tris (pH 7.8) 0.005 M EDTA 0.5% SDS	37–56°C	none required

[a]Pronase is a mixture of serine and acid proteases isolated from *Streptomyces griseus*.

[b]Self-digestion eliminates contamination with DNAase and RNAase. Self-digested pronase is prepared by dissolving powdered pronase in 10 mM Tris · Cl (pH 7.5), 10 mM NaCl to a final concentration of 20 mg/ml and incubating for 1 hour at 37°C. Store the self-digested pronase in small aliquots at −20°C in tightly capped tubes.

[c]Proteinase K is a highly active protease of the subtilisin type that is purified from the mold *Tritirachium album* Limber. The enzyme has two binding sites for Ca^{++}, which lie some distance from the active site and are not directly involved in the catalytic mechanism. However, when Ca^{++} is removed from the enzyme, approximately 80% of the catalytic activity is lost because of long-range structural changes (Bajorath et al. 1989). Because the residual activity is usually sufficient to degrade proteins that commonly contaminate preparations of nucleic acids, digestion with proteinase K is usually carried out in the presence of EDTA (to inhibit the action of Mg^{++}-dependent nucleases). However, to digest highly resistant proteins such as keratin, it may be necessary to use a buffer containing 1 mM Ca^{++} and no EDTA. At the end of the digestion, the Ca^{++} should be chelated by addition of EGTA (pH 8.0) to a final concentration of 2 mM before the nucleic acids are purified.

ANTIBIOTICS

TABLE A.1 Antibiotic Solutions

	Stock solution[a]		Working concentration	
	concentration	storage	stringent plasmids	relaxed plasmids
Ampicillin	50 mg/ml in H_2O	−20°C	20 μg/ml	60 μg/ml
Carbenicillin	50 mg/ml in H_2O	−20°C	20 μg/ml	60 μg/ml
Chloramphenicol	34 mg/ml in ethanol	−20°C	25 μg/ml	170 μg/ml
Kanamycin	10 mg/ml in H_2O	−20°C	10 μg/ml	50 μg/ml
Streptomycin	10 mg/ml in H_2O	−20°C	10 μg/ml	50 μg/ml
Tetracycline[b]	5 mg/ml in ethanol	−20°C	10 μg/ml	50 μg/ml

[a]Stock solutions of antibiotics dissolved in H_2O should be sterilized by filtration through a 0.22-micron filter. Antibiotics dissolved in ethanol need not be sterilized. Store solutions in light-tight containers.

[b]Magnesium ions are antagonists of tetracycline. Use media without magnesium salts (e.g., LB medium) for selection of bacteria resistant to tetracycline.

COMMONLY USED BUFFERS

TE

pH 7.4
10 mM Tris · Cl (pH 7.4)
1 mM EDTA (pH 8.0)

pH 7.6
10 mM Tris · Cl (pH 7.6)
1 mM EDTA (pH 8.0)

pH 8.0
10 mM Tris · Cl (pH 8.0)
1 mM EDTA (pH 8.0)

STE (also called TEN)

0.1 M NaCl
10 mM Tris · Cl (pH 8.0)
1 mM EDTA (pH 8.0)

STET

0.1 M NaCl
10 mM Tris · Cl (pH 8.0)
1 mM EDTA (pH 8.0)
5% Triton X-100

TNT

10 mM Tris · Cl (pH 8.0)
150 mM NaCl
0.05% Tween 20