COMMONLY USED REAGENTS AND EQUIPMENT

Commonly Used Reagents

This section describes the preparation of buffers and reagents commonly used in this manual. For a list of where to find formulations of media, please refer to *APPENDIX 2C*. When preparing solutions, use deionized, distilled water and reagents of the highest available grade. Sterilization—by filtration through a 0.22-μm filter or by autoclaving—is recommended for most solutions stored at room temperature and is essential for cell culture applications. Where storage conditions are not specified, store up to 6 months at room temperature. Discard any reagent that shows evidence of contamination, precipitation, or discoloration.

CAUTION: Handle strong acids and bases with care. See *UNIT 1A.3* for more information concerning the use of hazardous chemicals.

Acid, concentrated stock solutions

See Table A.2A.1.

Acid precipitation solution

1 M HCl (Table A.2A.1)
0.1 M sodium pyrophosphate

Nucleic acids can also be precipitated with a 10% (w/v) solution of trichloroacetic acid (TCA; see recipe); however, this recipe is cheaper, easier to prepare, and just as efficacious.

Table A.2A.1 Molarities and Specific Gravities of Concentrated Acids and Bases[a]

Acid/base	Mol. wt.	% by weight	Molarity (approx.)	Specific gravity	1 M solution (ml/liter)
Acetic acid (glacial)[b]	60.05	99.6	17.4	1.05	57.5
Ammonium hydroxide	35.0	28	14.8	0.90	67.6
Formic acid[b]	46.03	90	23.6	1.205	42.4
		98	25.9	1.22	38.5
Hydrochloric acid	36.46	36	11.6	1.18	85.9
Nitric acid	63.01	70	15.7	1.42	63.7
Perchloric acid	100.46	60	9.2	1.54	108.8
		72	12.2	1.70	82.1
Phosphoric acid[b]	98.00	85	14.7	1.70	67.8
Sulfuric acid	98.07	98	18.3	1.835	54.5

[a] *CAUTION:* Handle strong acids and bases carefully (see *UNIT 1A.3*).
[b] Also see Table A.2A.3.

Current Protocols in Microbiology (2005) A.2A.1-A.2A.15

Alsever's solution

20.5 g dextrose (114 mM)
7.9 g sodium citrate·$2H_2O$ (27 mM)
4.2 g NaCl (71 mM)
H_2O to 1 liter
Adjust to pH 6.1 with 1 M citric acid (Table A.2A.3) and filter sterilize
Store indefinitely at 4°C

Ammonium hydroxide, concentrated stock solution

See Table A.2A.1

Ammonium acetate, 10 M

Dissolve 385.4 g ammonium acetate in 150 ml H_2O
Add H_2O to 500 ml

Ammonium sulfate, saturated

76 g ammonium sulfate
100 ml H_2O
Heat with stirring to just below boiling point
Let stand overnight at room temperature

ATP, 100 mM

1 g ATP (adenosine triphosphate)
12 ml H_2O
Adjust pH to 7.0 with 4 M NaOH
Adjust volume to 16.7 ml with H_2O
Store in aliquots indefinitely at −20°C

Base, concentrated stock solutions

See Table A.2A.1.

BBS (BES-buffered solution), 2×

50 mM *N*,*N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES; Calbiochem)
280 mM NaCl
1.5 mM sodium phosphate buffer, pH 6.95 (see recipe)
800 ml H_2O
Adjust pH to 6.95 with room temperature 1 N NaOH
H_2O to 1 liter
Filter sterilize through a 0.45-μm nitrocellulose filter (Nalgene)
Store in aliquots at −20°C (can be frozen and thawed repeatedly)

The pH of this solution is critical (pH 6.95 to 6.98). When a new batch of 2× BES buffer is prepared, its pH should be checked against a reference stock prepared (and tested) earlier.

BCIP, 5% (w/v)

Dissolve 0.5 g 5-bromo-4-chloro-3-indolyl phosphate disodium salt (stored at −20°C) in 10 ml of 100% dimethylformamide (DMF). Store wrapped in aluminum foil up to 6 months at 4°C.

The BCIP may not dissolve completely. Vortex the solution immediately before use and pipet with a wide-mouth pipet tip.

Discard solution if it turns pinkish.

BSA (bovine serum albumin), 10% (w/v)

Dissolve 10 g BSA (e.g., Sigma) in 100 ml H_2O. Filter sterilize using a low-protein-binding 0.22-μm filter. Store indefinitely at 4°C.

Lower-concentration stock solutions (e.g., 1%), which are useful for various applications, can be made by diluting 10% stock appropriately with sterile water.

BSA is available in various forms that differ in fraction of origin, preparation, purity, pH, and cost; the most commonly used is fraction V. Use the form that is appropriate for the application; this may need to be optimized empirically.

$CaCl_2$, 1 M

147 g $CaCl_2 \cdot 2H_2O$
H_2O to 1 liter

Carbonate buffer

1.6 g Na_2CO_3 (15 mM final)
2.9 g $NaHCO_3$ (35 mM final)
0.2 g NaN_3 (3.1 mM final)
H_2O to 1 liter
Adjust to pH 9.5

CAUTION: Sodium azide is poisonous; follow appropriate precautions for handling, storage, and disposal.

Denhardt solution, 100×

10 g Ficoll 400
10 g polyvinylpyrrolidone
10 g bovine serum albumin (Pentax Fraction V; Miles Laboratories)
H_2O to 500 ml
Filter sterilize and store at −20°C in 25-ml aliquots

DEPC (diethylpyrocarbonate)-treated solutions

Add 0.2 ml DEPC to 100 ml of the solution to be treated. Shake vigorously to dissolve the DEPC. Autoclave the solution to inactivate the remaining DEPC.

CAUTION: Wear gloves and use a fume hood when using DEPC, as it is a suspected carcinogen.

Many investigators keep the solutions they use for RNA work separate to ensure that "dirty" pipets do not go into them.

Do not treat solutions containing Tris with DEPC, as Tris inactivates the DEPC.

DNase I, RNase-free (1 mg/ml)

Prepare a solution of 0.1 M iodoacetic acid plus 0.15 M sodium acetate and adjust pH to 5.3. Filter sterilize. Add sterile solution to lyophilized RNase-free DNase I (e.g., Worthington) to give a final concentration of 1 mg/ml. Heat 40 min at 55°C and then cool. Add 1 M $CaCl_2$ to a final concentration of 5 mM. Store at −80°C in small aliquots.

dNTPs: dATP, dTTP, dCTP, and dGTP

Concentrated stocks: Purchase deoxyribonucleoside triphosphates (dNTPs) from a commercial supplier either as ready-made 100 mM solutions (the preferred form for shipping and storage) or in lyophilized form. If purchased lyophilized, dissolve dNTPs in deionized water to an expected concentration of 30 mM, then adjust to

continued

Table A.2A.2 Molar Extinction Coefficients of DNA Bases

Base	Molar extinction coefficient (ε)[a]
Adenine	15,200
Cytosine	7050
Guanosine	12,010
Thymine	8400

[a] 1 M solution measured at 260 nm; see Wallace and Miyada (1987).

pH 7.0 with 1 M NaOH (to prevent acid-catalyzed hydrolysis). Determine the actual concentration of each dNTP by UV spectrophotometry at 260 nm, referring to the molar extinction coefficients given in Table A.2A.2.

Working solutions: Prepare working solutions of desired concentration (commonly 2 mM) for each dNTP by diluting concentrated stocks appropriately. Remember that the molarity of the 3dNTP and 4dNTP mixes refers to the concentration of *each* precursor present in the solution.

4dNTP mixes: Prepare mixed dNTP solutions containing equimolar amounts of all four DNA precursors; e.g.:

2 mM 4dNTP mix: 2 mM *each* dATP, dTTP, dCTP, and dGTP
1.25 mM 4dNTP mix: 1.25 mM *each* dATP, dTTP, dCTP, and dGTP.

3dNTP mixes: Prepare stocks lacking one particular dNTP but containing equimolar amounts of the remaining three precursors; e.g.:

2 mM 3dNTP mix (minus dATP): 2 mM *each* dTTP, dCTP, and dGTP.

Store dNTPs and dNTP mixtures as aliquots at −20°C (stable for ≤1 year).

DPBS (Dulbecco's phosphate-buffered saline)

8.00 g NaCl (0.137 M)
0.20 g KCl (2.7 mM)
0.20 g KH_2PO_4 (1.1 mM)
0.10 g $MgCl_2 \cdot 6H_2O$ (0.5 mM)
2.16 g $Na_2HPO_4 \cdot 7H_2O$ (8.1 mM)
0.10 g anhydrous $CaCl_2$ (0.9 mM)
H_2O to 1 liter

DPBS may be made or purchased without Ca^{2+} and Mg^{2+} (CMF-DPBS). These components are optional and usually have no effect on an experiment; in a few cases, however, their presence may be detrimental. Consult individual protocols to see if the presence or absence of these components is recommended.

Also see recipes for phosphate-buffered saline (PBS) and phosphate-buffered saline containing potassium (KPBS).

DTT (dithiothreitol), 1 M

Dissolve 1.55 g DTT in 10 ml water
Filter sterilize
Store in aliquots at −20°C

EDTA (ethylenediaminetetraacetic acid), 0.5 M (pH 8.0)

Dissolve 186.1 g disodium EDTA dihydrate in 700 ml water. Adjust pH to 8.0 with 10 M NaOH (~50 ml; add slowly). Add water to 1 liter and filter sterilize.

Begin titrating before the sample is completely dissolved. EDTA, even in the disodium salt form, is difficult to dissolve at this concentration unless the pH is increased to between 7 and 8.

Ethidium bromide staining solution

Concentrated stock (10 mg/ml): Dissolve 0.2 g ethidium bromide in 20 ml water. Mix well and store at 4°C in the dark or in a foil-wrapped bottle. Do not sterilize.

Working solution: Dilute stock to 0.5 μg/ml or other desired concentration in electrophoresis buffer (e.g., 1× TBE or TAE; see recipes) or in water.

Ethidium bromide working solution is used to stain agarose gels to permit visualization of nucleic acids under UV light. Gels should be placed in a glass dish containing sufficient working solution to cover them and shaken gently or allowed to stand for 10 to 30 min. If necessary, gels can be destained by shaking in electrophoresis buffer or water for an equal length of time to reduce background fluorescence and facilitate visualization of small quantities of DNA.

Alternatively, a gel can be run directly in ethidium bromide by using working solution (made with electrophoresis buffer) as the solvent and running buffer for the gel.

CAUTION: Ethidium bromide is a mutagen and must be handled carefully. See UNIT 1A.3 for more information.

FBS (fetal bovine serum)

Thaw purchased fetal bovine serum (shipped on dry ice and kept frozen until needed). Store 3 to 4 weeks at 4°C. If FBS is not to be used within this time, aseptically divide into smaller aliquots and refreeze until used. Store ≤1 year at −20°C.

FBS is shipped on dry ice and kept frozen until needed. Repeated thawing and refreezing should be avoided, as it may cause denaturation of the serum.

FBS, heat inactivated

Heat FBS (see recipe) 30 min to 1 hr in a 56°C water bath with periodic gentle swirling during the first 10 to 15 min to ensure uniform heating.

Treating FBS in this manner inactivates complement protein and thus prevents an immunological reaction against cultured cells, making it useful for a variety of purposes.

This reagent can also be purchased commercially.

Formamide loading buffer, 2×

Prepare in deionized formamide
0.05% (w/v) bromphenol blue
0.05% (w/v) xylene cyanol FF
20 mM EDTA
Do not sterilize
Store at −20°C

HBSS (Hanks' balanced salt solution)

0.40 g KCl (5.4 mM final)
0.09 g $Na_2HPO_4 \cdot 7H_2O$ (0.3 mM final)
0.06 g KH_2PO_4 (0.4 mM final)
0.35 g $NaHCO_3$ (4.2 mM final)

continued

0.14 g $CaCl_2$ (1.3 mM final)
0.10 g $MgCl_2 \cdot 6H_2O$ (0.5 mM final)
0.10 g $MgSO_4 \cdot 7H_2O$ (0.6 mM final)
8.0 g NaCl (137 mM final)
1.0 g D-glucose (5.6 mM final)
0.2 g phenol red (0.02%; optional)
Add H_2O to l liter and adjust pH to 7.4 with 1 M HCl (Table A.2A.1) or 1 M NaOH
Filter sterilize and store up to 1 month at 4°C

HBSS may be made or purchased without Ca^{2+} and Mg^{2+} (CMF-HBSS). These components are optional and usually have no effect on an experiment; in a few cases, however, their presence may be detrimental. Consult individual protocols to see if the presence or absence of these components is recommended.

Bottles should be kept tightly closed to prevent CO_2 loss and subsequent alkalinization.

HCl, 1 M

Mix the following in order:
913.8 ml H_2O
86.2 ml concentrated HCl (Table A.2A.1)

HeBS (HEPES-buffered saline) solution, 2×

16.4 g NaCl
11.9 g HEPES acid (Table A.2A.3)
0.21 g Na_2HPO_4
800 ml H_2O
Titrate to pH 7.05 with 5 M NaOH
Add H_2O to 1 liter
Filter sterilize through a 0.45-μm nitrocellulose filter
Store in 50-ml aliquots at −20°C

If the solution is to be used for transfection, the pH should be between 7.05 and 7.12, and should be tested for transfection efficiency.

KCl, 1 M

74.6 g KCl
H_2O to 1 liter

2-ME (2-mercaptoethanol), 50 mM

Prepare 1 M stock:
0.5 ml 14.3 M 2-ME
6.6 ml H_2O
Prepare 50 mM stock:
5 ml 1 M 2-ME
95 ml H_2O
Store at 4°C

$MgCl_2$, 1 M

20.3 g $MgCl_2 \cdot 6H_2O$
H_2O to 100 ml

$MgSO_4$, 1 M

24.6 g $MgSO_4 \cdot 7H_2O$
H_2O to 100 ml

MOPS buffer

0.2 M MOPS [3-(*N*-morpholino)-propanesulfonic acid], pH 7.0 (Table A.2A.3)
0.5 M sodium acetate
0.01 M EDTA
Store in the dark and discard if it turns yellow

NaCl, 5 M

292 g NaCl
H_2O to 1 liter

NaOH, 10 M

Dissolve 400 g NaOH in 450 ml H_2O
Add H_2O to 1 liter

PCR amplification buffer, 10×

500 mM KCl
100 mM Tris·Cl, pH 8.3 (see recipe)
x mM $MgCl_2$
0.1% (w/v) gelatin
Store in aliquots at −20°C

This solution can be sterilized by autoclaving. Alternatively, it can be made from sterile water and stock solutions, and the sterilization omitted.

15 mM $MgCl_2$ is the concentration (x) used for most PCR reactions. However, the optimal concentration depends on the sequence and primer of interest and may have to be determined experimentally.

Phenol, buffered

Add 0.5 g of 8-hydroxyquinoline to a 2-liter glass beaker containing a stir bar. Gently pour in 500 ml liquefied phenol or melted crystals of redistilled phenol (melt in a water bath at 65°C). The phenol will turn yellow due to the 8-hydroxyquinoline, which is added as an antioxidant. Add 500 ml of 50 mM Tris base. Cover the beaker with aluminum foil and stir 10 min at room temperature using a magnetic stirrer on low speed. Let phases separate at room temperature. Gently decant the top (aqueous) phase into a suitable waste receptacle (*UNIT 1A.3*). Remove what cannot be decanted with a 25-ml glass pipet and a suction bulb. Add 500 ml of 50 mM Tris·Cl, pH 8.0 (see recipe). Repeat equilibration with 500 ml of 50 mM Tris·Cl, pH 8.0, twice. Check the pH of the phenol phase with indicator paper to determine if it is 8.0. If it is not, repeat equilibration until this pH is obtained. Add 250 ml of 50 mM Tris·Cl, pH 8.0, or TE buffer, pH 8.0 (see recipe), and store at 4°C in brown glass bottles or clear glass bottles wrapped in aluminum foil.

CAUTION: Phenol can cause severe burns to skin and damage clothing. Gloves, safety glasses, and a laboratory coat should be worn whenever working with phenol, and all manipulations should be carried out in a fume hood. A glass receptacle should be available exclusively for disposing of used phenol and chloroform (see UNIT 1A.3).

Phenol prepared with 8-hydroxyquinoline as an antioxidant can be stored ≤2 months at 4°C.

Phenol must be redistilled before use, because oxidation products of phenol can damage and introduce breaks into nucleic acid chains. Redistilled phenol is commercially available. Regardless of the source, phenol must be buffered before use.

Table A.2A.3 pK_a Values and Molecular Weights for Some Common Biological Buffers[a]

Name	Chemical formula or IUPAC name	pK_a	Useful pH range	Mol. wt. (g/mol)
Phosphoric acid[b]	H_3PO_4	2.12 (pK_{a1})	—	98.00
Citric acid[c]	$C_6H_8O_7$ (H_3Cit)	3.06 (pK_{a1})	—	192.1
Formic acid[b]	HCOOH	3.75	—	46.03
Succinic acid	$C_4H_6O_4$	4.19 (pKa_1)	—	118.1
Citric acid[c]	$C_6H_7O_7^-$ (H_2Cit^-)	4.74 (pKa_2)	—	
Acetic acid[b]	CH_3COOH	4.75	—	60.05
Citric acid[c]	$C_6H_6O_7^-$ ($HCit_2^-$)	5.40 (pK_{a3})	—	
Succinic acid	$C_4H_5O_4^-$	5.57 (pK_{a2})	—	
MES	2-(*N*-Morpholino]ethanesulfonic acid	6.15	5.5-6.7	195.2
Bis-Tris	bis(2-Hydroxyethyl)iminotris (hydroxymethyl)methane	6.50	5.8-7.2	209.2
ADA	*N*-(2-Acetamido)-2-iminodiacetic acid	6.60	6.0-7.2	190.2
PIPES	Piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)	6.80	6.1-7.5	302.4
ACES	*N*-(Carbamoylmethyl)-2-amino-ethanesulfonic acid	6.80	6.1-7.5	182.2
Imidazole	1,3-Diaza-2,4-cyclopentadiene	7.00	—	68.08
Diethylmalonic acid	$C_7H_{12}O_4$	7.20	—	160.2
MOPS	3-(*N*-Morpholino)propanesulfonic acid	7.20	6.5-7.9	209.3
Sodium phosphate, monobasic	NaH_2PO_4	7.21 (pK_{a2})	—	120.0
Potassium phosphate, monobasic	KH_2PO_4	7.21 (pK_{a2})	—	136.1
TES	*N*-tris(Hydroxymethyl)methyl-2-aminoethanesulfonic acid	7.40	6.8-8.2	229.3
HEPES	*N*-(2-Hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid)	7.55	6.8-8.2	238.3
HEPPSO	*N*-(2-Hydroxyethyl)piperazine-*N*′-(2-hydroxypropanesulfonic acid)	7.80	7.1-8.5	268.3
Glycinamide·HCl	$C_2H_6N_2O$·HCl	8.10	7.4-8.8	110.6
Tricine	*N*-tris(Hydroxymethyl)methylglycine	8.15	7.4-8.8	179.2
Glycylglycine	$C_4H_8N_2O_3$	8.20	7.5-8.9	132.1
Tris	Tris(hydroxymethyl)aminomethane	8.30	7.0-9.0	121.1
Bicine	*N*,*N*-bis(2-Hydroxyethyl)glycine	8.35	7.6-9.0	163.2
Boric acid	H_3BO_3	9.24	—	61.83
CHES	2-(*N*-Cyclohexylamino)ethane-sulfonic acid	9.50	8.6-10.0	207.3
CAPS	3-(Cyclohexylamino)-1-propane-sulfonic acid	10.40	9.7-11.1	221.3

continued

Table A.2A.3 pK_a Values and Molecular Weights for Some Common Biological Buffers[a] , *continued*

Name	Chemical formula or IUPAC name	pK_a	Useful pH range	Mol. wt. (g/mol)
Sodium phosphate, dibasic	Na_2HPO_4	12.32 (pK_{a3})	—	142.0
Potassium phosphate, dibasic	K_2HPO_4	12.32 (pK_{a3})	—	174.2

[a]Some data reproduced from Buffers: A Guide for the Preparation and Use of Buffers in Biological Systems (Mohan, 1997) with permission of Calbiochem.

[b]See Table A.2A.1 for more information.

[c]Available as a variety of salts, e.g., ammonium, lithium, sodium.

Phenol/chloroform/isoamyl alcohol, 25:24:1 (v/v/v)

25 vol buffered phenol (bottom yellow phase of stored solution; see recipe)
24 vol chloroform
1 vol isoamyl alcohol
Store up to 2 months at 4°C

Phosphate-buffered saline (PBS)

0.23 g NaH_2PO_4 (anhydrous; 1.9 mM)
1.15 g Na_2HPO_4 (anhydrous; 8.1 mM)
9.00 g NaCl (154 mM)
Add H_2O to 900 ml
Adjust to desired pH (7.2 to 7.4) using 1 M NaOH or 1 M HCl (see recipe and Table A.2A.1)
Add H_2O to 1 liter

Also see recipes for phosphate-buffered saline containing potassium (KPBS) and Dulbecco's phosphate-buffered saline (DPBS).

Phosphate-buffered saline containing potassium (KPBS)

8.00 g NaCl (0.137 M)
0.20 g KCl (2.7 mM)
0.24 g KH_2PO_4 (1.4 mM)
1.44 g Na_2HPO_4 (0.01 M)
H_2O to 1 liter

Also see recipes for phosphate-buffered saline (PBS) and Dulbecco's phosphate-buffered saline (DPBS).

PMSF (phenylmethylsulfonyl fluoride), 100 mM

Dissolve 0.174 g PMSF in 10 ml of 100% ethanol, isopropanol, or methanol. Store in aliquots up to 2 years at −20°C.

CAUTION: Phenylmethylsulfonyl fluoride is toxic.

Make fresh dilutions from the alcohol stock for each use, because the half-life of PMSF in aqueous solution is <30 min at room temperature and a few hours on ice.

If PMSF is being added to a solution without detergent, the solution should be stirred vigorously during addition because PMSF has a tendency to form an insoluble precipitate in aqueous solution.

Potassium acetate buffer, 0.1 M

Solution A: 11.55 ml glacial acetic acid (Tables A.2A.1 and A.2A.3) per liter (0.2 M) in water.

continued

Table A.2A.4 Preparation of 0.1 M Sodium and Potassium Acetate Buffers[a]

Desired pH	Solution A (ml)	Solution B (ml)
3.6	46.3	3.7
3.8	44.0	6.0
4.0	41.0	9.0
4.2	36.8	13.2
4.4	30.5	19.5
4.6	25.5	24.5
4.8	20.0	30.0
5.0	14.8	35.2
5.2	10.5	39.5
5.4	8.8	41.2
5.6	4.8	45.2

[a]Adapted with permission from CRC (1975).

Solution B: 19.6 g potassium acetate ($KC_2H_3O_2$) per liter (0.2 M) in water.

Referring to Table A.2A.4 for desired pH, mix the indicated volumes of solutions A and B, then dilute with water to 100 ml. Filter sterilize if necessary. Store up to 3 months at room temperature.

This may be made as a 5- or 10-fold concentrate by scaling up the amount of sodium acetate in the same volume. Acetate buffers show concentration-dependent pH changes, so check the pH by diluting an aliquot of concentrate to the final concentration.

To prepare buffers with pH intermediate between the points listed in Table A.2A.4, prepare the closest higher pH, then titrate with solution A.

Potassium phosphate buffer, 0.1 M

Solution A: 27.2 g KH_2PO_4 (Table A.2A.3) per liter (0.2 M final) in water

Solution B: 34.8 g K_2HPO_4 (Table A.2A.3) per liter (0.2 M final) in water

Referring to Table A.2A.5 for desired pH, mix the indicated volumes of solutions A and B, then dilute with water to 200 ml. Filter sterilize if necessary. Store up to 3 months at room temperature.

This buffer may be made as a 5- or 10-fold concentrate simply by scaling up the amount of potassium phosphate in the same final volume. Phosphate buffers show concentration-dependent changes in pH, so check the pH of the concentrate by diluting an aliquot to the final concentration.

To prepare buffers with pH intermediate between the points listed in Table A.2A.5, prepare closest higher pH, then titrate with solution A.

RNase A stock solution, DNase-free, 2 mg/ml

Dissolve RNase A (e.g., Sigma) in DEPC-treated H_2O (see recipe) to 2 mg/ml. Boil 10 min in a 100°C water bath. Store up to 1 year at 4°C.

The activity of the enzyme varies from lot to lot; therefore, prepare several 10-ml aliquots of each dilution to facilitate standardization.

Table A.2A.5 Preparation of 0.1 M Sodium and Potassium Phosphate Buffers[a]

Desired pH	Solution A (ml)	Solution B (ml)
5.7	93.5	6.5
5.8	92.0	8.0
5.9	90.0	10.0
6.0	87.7	12.3
6.1	85.0	15.0
6.2	81.5	18.5
6.3	77.5	22.5
6.4	73.5	26.5
6.5	68.5	31.5
6.6	62.5	37.5
6.7	56.5	43.5
6.8	51.0	49.0
6.9	45.0	55.0
7.0	39.0	61.0
7.1	33.0	67.0
7.2	28.0	72.0
7.3	23.0	77.0
7.4	19.0	81.0
7.5	16.0	84.0
7.6	13.0	87.0
7.7	10.5	90.5
7.8	8.5	91.5
7.9	7.0	93.0
8.0	5.3	94.7

[a]Adapted by permission from CRC (1975).

Saline, 0.9%

9 g NaCl (154 mM final; 0.9% w/v)
H_2O to 1 liter

Saponin, 10% (w/v)

Dissolve 1 g saponin in 10 ml PBS (see recipe)
Store in 500-µl aliquots at −20°C

Once thawed, the 10% solution is stable for several months when stored at 4°C.

SDS, 20% (w/v)

Dissolve 20 g sodium dodecyl sulfate (SDS; also known as sodium lauryl sulfate, SLS) in H_2O in a total volume of 100 ml with stirring. Filter sterilize using a 0.45-µm filter.

It may be necessary to heat the solution slightly to fully dissolve the powder.

Table A.2A.6 Preparation of SDS Sample Buffer

Ingredient	2×	4×	Final conc. in 1× buffer
0.5 M Tris·Cl, pH 6.8[a]	2.5 ml	5.0 ml	62.5 mM
SDS	0.4 g	0.8 g	2% (w/v)
Glycerol	2.0 ml	4.0 ml	10% (v/v)
Bromphenol blue	20 mg	40 mg	0.1% (w/v)
2-Mercaptoethanol[a,b,c]	400 µl	800 µl	~300 mM
H_2O	to 10 ml	to 10 ml	—

[a] See recipe.
[b] Alternatively, dithiothreitol (DTT; see recipe), at a final concentration of 100 mM, can be substituted for 2-mercaptoethanol.
[c] Add just before use.

SDS sample buffer

See Table A.2A.6

SED (standard enzyme diluent)

20 mM Tris·Cl, pH 7.5 (see recipe)
500 µg/ml bovine serum albumin (Pentax Fraction V)
10 mM α-mercaptoethanol
Store at 4°C for up to 1 month

Sodium acetate, 3 M

Dissolve 408 g sodium acetate trihydrate ($NaC_2H_3O_2 \cdot 3H_2O$) in 800 ml H_2O
Adjust pH to 4.8, 5.0, or 5.2 (as desired) with 3 M acetic acid
Add H_2O to 1 liter
Filter sterilize

Sodium acetate buffer, 0.1 M

Solution A: 11.55 ml glacial acetic acid (Tables A.2A.1 and A.2A.3) per liter (0.2 M) in water.

Solution B: 27.2 g sodium acetate ($NaC_2H_3O_2 \cdot 3H_2O$) per liter (0.2 M) in water.

Referring to Table A.2A.4 for desired pH, mix the indicated volumes of solutions A and B, then dilute with water to 100 ml. Filter sterilize if necessary. Store up to 3 months at room temperature.

This may be made as a 5- or 10-fold concentrate by scaling up the amount of sodium acetate in the same volume. Acetate buffers show concentration-dependent pH changes, so check the pH by diluting an aliquot of concentrate to the final concentration.

To prepare buffers with pH intermediate between the points listed in Table A.2A.4, prepare closest higher pH, then titrate with solution A.

Sodium phosphate buffer, 0.1 M

Solution A: 27.6 g $NaH_2PO_4 \cdot H_2O$ (Table A.2A.3) per liter (0.2 M final) in water

Solution B: 53.65 g $Na_2HPO_4 \cdot 7H_2O$ (Table A.2A.3) per liter (0.2 M) in water

continued

Referring to Table A.2A.5 for desired pH, mix the indicated volumes of solutions A and B, then dilute with water to 200 ml. Filter sterilize if necessary. Store up to 3 months at room temperature.

This buffer may be made as a 5- or 10-fold concentrate by scaling up the amount of sodium phosphate in the same final volume. Phosphate buffers show concentration-dependent changes in pH, so check the pH by diluting an aliquot of the concentrate to the final concentration.

To prepare buffers with pH intermediate between the points listed in Table A.2A.5, prepare the closest higher pH, then titrate with solution A.

Silanized glassware

For smaller items: In a well-vented fume hood, place glassware or plasticware (e.g., tubes, tips) in a dedicated vacuum desiccator with an evaporating dish containing 1 ml dichlorodimethylsilane. Apply vacuum with an aspirator and allow ~50% of the liquid to evaporate (several minutes). Turn off aspirator and allow items to remain under vacuum for 30 min. Remove the lid and allow fumes to vent into the hood for ~30 min. If desired, autoclave silanized items.

Do not leave the desiccator attached to the vacuum pump. This will suck away the silane, minimizing deposition and damaging the pump.

For larger items: Silanize items that do not fit in a desiccator by briefly rinsing with or soaking in a solution of ~5% dichlorodimethylsilane in a volatile organic solvent (e.g., chloroform, heptane). Remove organic solvent by evaporation, allowing deposition of dichlorodimethylsilane.

This approach is particularly useful for treating glass plates for denaturing polyacrylamide sequencing gels.

Treatment of glassware, plasticware, or equipment with dichlorodimethylsilane introduces a short polymer of dimethylsiloxane onto its surface. Polydimethylsiloxane is silicone oil. Autoclaving or rinsing with water removes the reactive chlorosilane end of the dimethylsiloxane polymer generated by dichlorodimethylsilane.

CAUTION: Dichlorodimethylsilane vapors are toxic and highly flammable. Always perform in a fume hood.

SSC (sodium chloride/sodium citrate), 20×

Dissolve the following in 900 ml H_2O:
175 g NaCl (3 M final)
88 g trisodium citrate dihydrate (0.3 M final)
Adjust pH to 7.0 with 1 M HCl (see recipe and Table A.2A.1)
Adjust volume to 1 liter
Filter sterilize
Store up to 6 months at room temperature

SSPE (sodium chloride/sodium phosphate/EDTA), 20×

175.2 g NaCl
27.6 g $NaH_2PO_4 \cdot H_2O$
7.4 g disodium EDTA
800 ml H_2O
Adjust pH to 7.4 with 6 M NaOH, then bring volume to 1 liter with H_2O
Filter sterilize
Store up to 6 months at room temperature

The final sodium concentration of 20× SSPE is 3.2 M.

T4 DNA ligase buffer, 10×

500 mM Tris·Cl, pH 7.6 (see recipe)
100 mM $MgCl_2$
10 mM DTT
10 mM ATP
250 μg/ml BSA
Store in aliquots at −20°C

TAE buffer, 50×

242 g Tris base
57.1 ml glacial acetic acid (Tables A.2A.1 and A.2A.3)
37.2 g $Na_2EDTA{\cdot}2H_2O$ (2 mM)
H_2O to 1 liter

This solution does not normally need to be sterilized. The Tris base and acetic acid correspond to 40 mM Tris·acetate.

TBE (Tris/borate/EDTA) buffer, 10×

108 g Tris base (890 mM)
55 g boric acid (890 mM; Table A.2A.3)
960 ml H_2O
40 ml 0.5 M EDTA, pH 8.0 (20 mM final; see recipe)

TBS (Tris-buffered saline)

100 mM Tris·Cl, pH 7.5 (see recipe)
0.9% (w/v) NaCl
Store up to several months at 4°C

TCA (trichloroacetic acid), 100% (w/v)

500 g TCA
227 ml H_2O

TE (Tris/EDTA) buffer

10 mM Tris·Cl, pH 7.4, 7.5, or 8.0 (or other pH; see recipe)
1 mM EDTA, pH 8.0 (see recipe)

TEA (triethanolamine) solution

50 mM triethanolamine, pH ∼11.5
0.1% (v/v) Triton X-100
0.15 M NaCl
Add Triton X-100 from a 10% stock (see recipe).

Tris·Cl, 1 M

Dissolve 121 g Tris base in 800 ml H_2O
Adjust to desired pH with concentrated HCl (Table A.2A.1)
Adjust volume to 1 liter with H_2O
Filter sterilize if necessary
Store up to 6 months at 4°C or room temperature

Approximately 70 ml HCl is needed to achieve a pH 7.4 solution and ∼42 ml for a solution that is pH 8.0.

IMPORTANT NOTE*: The pH of Tris buffers changes significantly with temperature, decreasing approximately 0.028 pH units per 1°C. Tris-buffered solutions should be adjusted to the desired pH at the temperature at which they will be used. Because of the pK_a, Tris should not be used as a buffer below pH ∼7.2 or above pH ∼9.0 (see Table A.2A.3).*

Triton X-100, 10% (w/v)

1 g Triton X-100
H_2O to 10 ml
Stir to dissolve
Filter sterilize through a 0.45-μm filter
Store protected from light up to 6 months at room temperature

TTBS (Tween 20/TBS)

Dissolve 0.1% (w/v) polyoxyethylenesorbitan monolaurate (Tween 20) in TBS (see recipe). Store up to several months at 4°C.

Urea loading buffer, 2×

5 mg bromphenol blue (0.05% w/v)
5 mg (w/v) xylene cyanol FF (0.05% w/v)
4.8 g urea (8 M)
186 mg EDTA (50 mM)
H_2O to 10 ml
Do not sterilize
Store up to 6 months at room temperature

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