Commonly Used Reagents

BUFFERS AND STOCK SOLUTIONS

This collection describes the preparation of buffers and reagents used in the manipulation of proteins (see Table A.2E.1). When preparing solutions, use water purified using a Milli-Q apparatus (Millipore) or equivalent and reagents of the highest grade available. Sterilization—by filtration through a 0.22-µm filter—is recommended for most applications.

CAUTION: *Handle strong acids and bases carefully.*

Ammonium acetate, 10 M

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

Dithiothreitol (DTT), 1 M

Dissolve 15.45 g DTT in 100 ml H_2O
Store at −20°C

EDTA (ethylenediamine tetraacetic acid), 0.5 M (pH 8.0)

Dissolve 186.1 g $Na_2EDTA{\cdot}2H_2O$ in 700 ml H_2O
Adjust pH to 8.0 with 10 M NaOH (~50 ml)
Add H_2O to 1 liter

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, 10 mg/ml

Dissolve 0.2 g ethidium bromide in 20 ml H_2O
Mix well and store at 4°C in dark

CAUTION: *Ethidium bromide is a mutagen and must be handled carefully.*

Table A.2E.1 Molarities and Specific Gravities of Concentrated Acids and Bases

Acid/base	Molecular weight	% by weight	Molarity (approx.)	1 M solution (ml/liter)	Specific gravity
Acids					
Acetic acid (glacial)	60.05	99.6	17.4	57.5	1.05
Formic acid	46.03	90	23.6	42.4	1.205
		98	25.9	38.5	1.22
Hydrochloric acid	36.46	36	11.6	85.9	1.18
Nitric acid	63.01	70	15.7	63.7	1.42
Perchloric acid	100.46	60	9.2	108.8	1.54
		72	12.2	82.1	1.70
Phosphoric acid	98.00	85	14.7	67.8	1.70
Sulfuric acid	98.07	98	18.3	54.5	1.835
Bases					
Ammonium hydroxide	35.0	28	14.8	67.6	0.90
Potassium hydroxide	56.11	45	11.6	82.2	1.447
Potassium hydroxide	56.11	50	13.4	74.6	1.51
Sodium hydroxide	40.0	50	19.1	52.4	1.53

HCl, 1 M

Mix in the following order:
914.1 ml H_2O
85.9 ml concentrated HCl

KCl, 1 M

74.6 g KCl
H_2O to 1 liter

$MgCl_2$, 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

NaCl, 5 M

292 g NaCl
H_2O to 1 liter

NaOH, 10 M

Mix in the following order:
476 ml H_2O
524 ml 50% (v/v) NaOH
Alternatively:
Dissolve 400 g NaOH in 450 ml H_2O
Add H_2O to 1 liter

Phenol, buffered

8-hydroxyquinoline
Liquefied phenol, redistilled
50 mM Tris base (unadjusted pH ~10.5)
50 mM Tris·Cl, pH 8.0 (see recipe)
TE buffer, pH 8.0 (see recipe)

Add 0.5 g of 8-hydroxyquinoline to a 2-liter glass beaker containing a stir bar. Gently pour in 500 ml of liquefied phenol or melted crystals of redistilled phenol (melted 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 low speed with magnetic stirrer at room temperature. Let phases separate at room temperature. Gently decant the top (aqueous) phase into a suitable waste receptacle. 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. Repeat equilibration with 500 ml of 50 mM Tris·Cl, pH 8.0, twice. The pH of the phenol phase can be checked with indicator paper and should be 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, and store at 4°C in brown glass bottles or clear glass bottles wrapped in aluminum foil.

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, the phenol must be buffered before use.

CAUTION*: Phenol can cause severe burns to skin and damage clothing. Gloves, safety glasses, and a lab 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.*

Table A.2E.2 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 by permission from CRC, 1975.

Table A.2E.3 Preparation of 0.1 M Sodium and Potassium Phosphate Buffers[a]

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

[a]Adapted by permission from CRC, 1975.

Phosphate-buffered saline (PBS)

10× stock solution, 1 liter:
80 g NaCl
2 g KCl
11.5 g $Na_2HPO_4 \cdot 7H_2O$
2 g KH_2PO_4

Working solution, pH ~7.3:
137 mM NaCl
2.7 mM KCl
4.3 mM $Na_2HPO_4 \cdot 7H_2O$
1.4 mM KH_2PO_4

Potassium acetate buffer, 0.1 M

Solution A: 11.55 ml glacial acetic acid/liter (0.2 M)
Solution B: 19.6 g potassium acetate ($KC_2H_3O_2$)/liter (0.2 M)

Referring to Table A.2E.2 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H_2O to 100 ml.

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

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

Potassium phosphate buffer, 0.1 M

Solution A: 27.2 g KH_2PO_4 per liter (0.2 M)
Solution B: 45.6 g $K_2HPO_4 \cdot 3H_2O$ per liter (0.2 M)

Referring to Table A.2E.3 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H_2O to 200 ml.

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

SDS, 20%

Dissolve 20 g SDS (sodium dodecyl sulfate or sodium lauryl sulfate) in H_2O to 100 ml total with stirring (it may be necessary to heat the solution slightly to fully dissolve the powder). Filter sterilize using a 0.45-μm filter.

Sodium acetate, 3 M

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

Sodium acetate buffer, 0.1 M

Solution A: 11.55 ml glacial acetic acid/liter (0.2 M)
Solution B: 27.2 g sodium acetate ($NaC_2H_3O_2 \cdot 3H_2O$)/liter (0.2 M)

Referring to Table A.2E.2 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H_2O to 100 ml. (See Potassium acetate buffer recipe for further details.)

Sodium phosphate buffer, 0.1 M

Solution A: 27.6 g $NaH_2PO_4 \cdot H_2O$ per liter (0.2 M)
Solution B: 53.65 g $Na_2HPO_4 \cdot 7H_2O$ per liter (0.2 M)

Referring to Table A.2E.3 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H_2O to 200 ml. (See Potassium phosphate buffer recipe for further details.)

SSC (sodium chloride/sodium citrate), 20×

3 M NaCl (175 g/liter)
0.3 M Na_3citrate$\cdot 2H_2O$ (88 g/liter)
Adjust pH to 7.0 with 1 M HCl

TAE (Tris/acetate/EDTA) electrophoresis buffer

50× stock solution:	*Working solution, pH ~8.5:*
242 g Tris base	40 mM Tris·acetate
57.1 ml glacial acetic acid	2 mM $Na_2EDTA \cdot 2H_2O$
37.2 g $Na_2EDTA \cdot 2H_2O$	
H_2O to 1 liter	

TBE (Tris/borate/EDTA) electrophoresis buffer

10× stock solution, 1 liter:
108 g Tris base (890 mM)
55 g boric acid (890 mM)
40 ml 0.5 M EDTA, pH 8.0 (see recipe; 20 mM)

TE buffer

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

Tris·Cl [tris(hydroxymethyl)aminomethane], 1 M

Dissolve 121 g Tris base in 800 ml H_2O
Adjust to desired pH with concentrated HCl
Mix and add H_2O to 1 liter

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 the pK_a of Tris is 8.08, Tris should not be used as a buffer below pH ~7.2 or above pH ~9.0.*

LITERATURE CITED

Chemical Rubber Company. 1975. CRC Handbook of Biochemistry and Molecular Biology, Physical and Chemical Data, 3rd ed., Vol. 1. CRC Press, Boca Raton, Fla.