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LABORATORY STOCK SOLUTIONS AND EQUIPMENT

APPENDIX 2

Common Buffers and Stock Solutions

APPENDIX 2A

This section describes the preparation of buffers and reagents used in the manipulation of nucleic acids.

For preparation of acid and base stock solutions, see Tables A.2A.1 and A.2A.2 as well as individual recipes.

GENERAL GUIDELINES

When preparing solutions, use deionized, distilled water and (for most applications) reagents of the highest grade available. Sterilization is recommended for most applications and is generally accomplished by autoclaving. Materials with components that are volatile, altered or damaged by heat, or whose pH or concentration are critical should be sterilized by filtration through a 0.22-μm filter. In many cases such components are added from concentrated stocks after the solution has been autoclaved. Where specialized sterilization methods are required, this is indicated in the individual recipes.

CAUTION: It is important to follow laboratory safety guidelines and heed manufacturers' precautions when working with hazardous chemicals; consult institutional safety officers and appropriate references for further details.

STORAGE

Most simple stock solutions can be stored indefinitely at room temperature if reasonable care is exercised to keep them sterile; where more rigorous conditions are required, this is indicated in the individual recipes.

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

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

[a]*CAUTION:* Handle strong acids and bases carefully.

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SPECIAL CONSIDERATIONS FOR WORKING WITH RNA

RNA is susceptible to degradation by ribonucleases, which are ubiquitous, very stable, and generally require no cofactors to function. Therefore, it is very important when working with RNA to take precautions against RNase contamination.

1. Treat all water and salt solutions except those containing Tris with DEPC (diethylpyrocarbonate; see recipe below), which inactivates ribonucleases by covalent modification.
2. If possible, make separate stock solutions to use for working with RNA and keep separate to ensure that "dirty" pipets do not come in contact with them.
3. Bake glassware 4 hr at 150°C. Rinse plasticware in chloroform or use directly out of the package (when it is generally free from contamination). Autoclaving will not fully inactivate many RNases.
4. Wear clean disposable gloves that have not been worn in any potentially RNase-contaminated areas.

SPECIAL CONSIDERATIONS FOR PCR EXPERIMENTS

Because the polymerase chain reaction (PCR) is designed to detect very small amounts of DNA, only a few molecules of contaminating DNA will produce unwanted amplification products. Ideally, PCR should not be carried out in the same room where large quantities of DNA are handled. Even where such spatial separation is not practical, the following housekeeping procedures will help avoid contamination with extraneous DNA (H.D. Kay, pers. comm.).

1. Keep laboratory surfaces clean by swabbing with 5% to 10% chlorine bleach. Put fresh absorbent paper bench protectors on bench before beginning PCR.
2. Wear disposable gloves and change them frequently while setting up PCRs.
3. Use only sterile disposable plasticware.
4. Keep a separate set of pipetting devices for setting up PCRs. If possible, use these instruments only with cotton-plugged tips to minimize transfer of DNA by aerosol. A separate microcentrifuge for PCR work is also desirable.
5. Whenever possible, set up PCRs in a laminar-flow hood or Class II biological safety cabinet to help prevent contamination by airborne DNA particles. A UV light within the hood or cabinet will help inactivate contaminating DNA.
6. Handle microcentrifuge tubes aseptically. Do not touch the interior of the hinged cap; if this happens, discard the tube. Microcentrifuge tubes briefly before opening to pellet drops around the cap and help keep reagents and reaction mixtures away from potentially contaminating fingers. Have only one tube open at a time, and open each tube away from the remaining tubes. Hand-held microcentrifuge tube openers (e.g., USA/Scientific Plastics) are available to facilitate aseptic technique.
7. Include negative controls (i.e., no primer and no template) in all PCRs.

SELECTION OF BUFFERS

Table A.2A.2 reports pK_a values for some common buffers. Note that polybasic buffers, such as phosphoric acid and citric acid, have more than one useful pK_a value. When choosing a buffer, select a buffer material with a pK_a close to the desired working pH (at the desired concentration and temperature for use). In general, effective buffers have a range of approximately 2 pH units centered about the pK_a value. Ideally the dissociation

constant—and therefore the pH—should not shift with a change in concentration or temperature. If the shift is small, as for MES and HEPES, then a concentrated stock solution can be prepared and diluted without adjustment to the pH. Buffers containing phosphate or citrate, however, show a significant shift in pH with concentration change, and Tris buffers show a large change in pH with temperature. For convenience, concentrated stock solutions of these buffers can still be used, provided that a pH adjustment is made *after* any temperature and concentration adjustments. All adjustments to pH should be made using the appropriate base—usually NaOH or KOH, depending on the corresponding free counterion. Tetramethylammonium hydroxide can be used to prepare buffers without a mineral cation. Many common buffers are supplied both as a free acid or base and as the corresponding salt. By mixing precalculated amounts of each, a series of buffers with varying pH values can conveniently be prepared.

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

Name	Chemical formula or IUPAC name	pK_a	Useful pH range	MW (g/mol)
Phosphoric acid	H_3PO_4	2.12 (pK_{a1})	—	98.00
Citric acid[b]	$C_6H_8O_7$ (H_3Cit)	3.06 (pK_{a1})	—	192.1
Formic acid	HCOOH	3.75	—	46.03
Succinic acid	$C_4H_6O_4$	4.19 (pKa_1)	—	118.1
Citric acid[b]	$C_6H_7O_7^-$ (H_2Cit^-)	4.74 (pKa_2)	—	
Acetic acid	CH_3COOH	4.75	—	60.05
Citric acid[b]	$C_6H_6O_7^-$ ($HCit^{2-}$)	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	$C_2H_6N_2O{\cdot}HCl$	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

continued

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

Name	Chemical formula or IUPAC name	pK_a	Useful pH range	MW (g/mol)
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
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]Available as a variety of salts, e.g., ammonium, lithium, sodium.

RECIPES

Ammonium acetate, 10 M

Dissolve 385.4 g ammonium acetate in 150 mL H_2O
Add H_2O to 500 mL
Sterilize by filtration

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.

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. One may also try to have separate working area, pipettors, etc. to avoid contamination.

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

dNTPs: dATP, dTTP, dCTP, and dGTP

Concentrated stocks: Purchase deoxyribonucleoside triphosphates (dNTPs) from a commercial supplier (Pharmacia Biotech is recommended) 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 H_2O to an expected concentration of 30 mM, then adjust to 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, using the following extinction coefficients: adenine, $\varepsilon^{IM}_{260nm} = 15{,}200$; cytosine, $\varepsilon^{IM}_{260nm} = 7{,}050$; guanosine, $\varepsilon^{IM}_{260nm} = 12{,}010$; thymine, $\varepsilon^{IM}_{260nm} = 8{,}400$.

continued

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: For use in various molecular biology applications, prepare mixed dNTP solutions containing equimolar amounts of all four DNA precursors; e.g.:

2 mM 4dNTP mix: 2 mM each dATP, dTTP, dCTP, dGTP

1.25 mM 4dNTP mix: 1.25 mM each of dATP, dTTP, dCTP, dGTP.

3dNTP mixes: For use in radioactive labeling procedures, prepare similar 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 of dTTP, dCTP, dGTP.

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

DTT (dithiothreitol), 1 M

Dissolve 1.55 g DTT in 10 mL water and filter sterilize. Store in aliquots at −20°C.

Do not autoclave to sterilize.

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 L 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. Heating the solution may also help to dissolve EDTA.

Ethidium bromide solution

Concentrated stock (10 mg/mL): Dissolve 0.2 g ethidium bromide in 20 mL H_2O. Mix well and store at 4°C in 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) or water.

To use: 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 toxic and powerful mutagen. Gloves should be worn when working with solution or gel and a mask should be worn when weighing out solid. Keep separate solid and liquid waste containers for disposal of ethidium bromide–contaminated material.*

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

Gel loading buffer, 6×

0.25% (w/v) bromphenol blue
0.25% (w/v) xylene cyanol FF
40% (w/v) sucrose *or* 15% (w/v) Ficoll 400 *or* 30% (v/v) glycerol
Store at 4°C (room temperature if Ficoll is used)

This buffer does not need to be sterilized. Sucrose, Ficoll 400, and glycerol are essentially interchangeable in this recipe.

Other concentrations (e.g., 10×) can be prepared if more convenient.

HCl, 1 M

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

HEPES-buffered saline, 2×

90 mL H_2O
1.6 g NaCl (0.27 M)
74.6 mg KCl (10 mM)
21.3 mg Na_2HPO_4 (1.5 mM)
0.18 g glucose (10 mM)
1.07 g HEPES (45 mM)
Adjust pH to desired value (see Table A.2A.2) with 0.5 N NaOH
Adjust volume to 100 mL with H_2O
Filter sterilize
Store in aliquots indefinitely at −20°C

KCl, 1 M

74.6 g KCl
H_2O to 1 L

Kinase buffer, 10×

700 mM Tris·Cl, pH 7.4 (see recipe below)
100 mM $MgCl_2$ (see recipe below)
Store in aliquots indefinitely at room temperature

Discard solution if a precipitate forms.

A 10× kinase buffer is sold with commercially available kinase enzymes, but usually contains DTT. This buffer may be used as a substitute if the reaction must be done under nonreducing conditions.

$MgCl_2$, 1 M

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

$MgCl_2$ is extremely hygroscopic. Do not store opened bottles for long periods of time.

$MgSO_4$, 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 L

NaOH, 10 M

Dissolve 400 g NaOH in 450 mL H_2O
Add H_2O to 1 L

NBT (nitroblue tetrazolium chloride), 5% (w/v)

Dissolve 0.5 g NBT in 10 mL of 70% dimethylformamide (DMF). Store wrapped in aluminum foil up to 1 year at 4°C.

PBS (phosphate-buffered saline)

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 800 mL
Adjust pH as desired (usually to pH 7.4 with 1 M HCl)
Add H_2O to 1 L

Prepackaged PBS (pH 7.4), which is reconstituted by adding water, is commercially available from Sigma. This is very convenient if used in large quantities.

PCR amplification buffer, 10×

500 mM KCl
100 mM Tris·Cl, pH 8.3 (see recipe below)
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

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

Add 0.5 g of 8-hydroxyquinoline to a 2-L 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. (Aspiration also works well to remove the majority of the top phase.) 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. DNA will partition into organic phase at acidic pH; therefore pH must be >7.8. 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. If liquefied phenol is yellowish or pink when purchased, discard.

continued

CAUTION*: Phenol can cause severe burns to skin and damage clothing. If it contacts skin, wash with soap and water. Do NOT use ethanol. 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. Filter tips (available from Rainin Instruments) should be used when pipetting solutions to protect pipettors.*

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

Mix 25 vol buffered phenol (bottom phase; see recipe above) with 24 vol chloroform and 1 vol isoamyl alcohol. Store in brown glass bottle or in clear glass bottle wrapped in aluminum foil ≤2 months at 4°C.

To use: Mix 1:1 (v/v) with DNA sample solution to form an emulsion, then microcentrifuge 15 sec at maximum speed, room temperature. Assuming that the salt concentration in the DNA is <0.5 M, the DNA-containing aqueous phase will be on top; transfer this to a new tube, being careful not to disturb the interface. Reextract if necessary, and then extract a final time with 1 vol chloroform to remove residual phenol.

Alternatively, isoamyl alcohol may be omitted and a 1:1 mix of phenol and chloroform used instead. The isoamyl alcohol enhances the separation of the phases and reduces foaming, however.

Fuller details of phenol extraction are given in CPMB UNIT 2.1.

PMSF (phenylmethanesulfonyl fluoride), 10 mM

Dissolve 1.74 mg/mL PMSF in isopropyl alcohol. Store aliquots indefinitely at –20°C.

CAUTION: *PMSF is extremely toxic to the mucous membranes of the respiratory tract, the eyes, and the skin. It may be fatal if inhaled, swallowed, or absorbed through the skin. Gloves, safety glasses, and a lab coat should be worn when working with PMSF. Wash any contacted areas immediately with large volumes of water and discard contaminated clothing.*

PMSF stock solutions can be made up to 17.4 mg/mL (100 mM) if necessary. Note that PMSF is inactivated in aqueous solutions; the rate of inactivation increases with both temperature and pH.

Methods for disposal and detection of PMSF can be found in Lunn (2000).

Potassium acetate buffer, 0.1 M

Solution A: 11.55 mL glacial acetic acid per L (0.2 M) in water.

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

continued

Table A.2A.3 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.2A.4 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).

Potassium acetate buffer, 0.1 M

Solution A: 11.55 mL glacial acetic acid per L (0.2 M) in water.

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

Referring to Table A.2A.3 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.3, prepare closest higher pH, then titrate with solution A.

Potassium phosphate buffer, 0.1 M

Solution A: 27.2 g KH_2PO_4 per L (0.2 M final) in water.

Solution B: 34.8 g K_2HPO_4 per L (0.2 M final) 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 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.4, prepare closest higher pH, then titrate with solution A.

SDS, 20% (w/v)

Dissolve 20 g SDS (sodium dodecyl sulfate or sodium lauryl sulfate) in water to 100 mL total volume with stirring. Filter sterilize using a 0.45-µm filter.

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

SDS sample buffer

See Table A.2A.5.

Table A.2A.5 Preparation of SDS Sample Buffer[a]

Ingredient	2×	4×	Final conc. in 1× buffer
0.5 M Tris·Cl, pH 6.8[b]	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[c,d]	400 µL	800 µL	~300 mM
H_2O	to 10 mL	to 10 mL	—

[a]Can be divided into aliquots and stored several months at room temperature (after addition of 2-ME); when solution turns yellow/green, discard.
[b]See recipe below.
[c]Alternatively, dithiothreitol (DTT), at a final concentration of 100 mM, can be substituted for 2-mercaptoethanol (2-ME).
[d]Add just before use.

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 (*APPENDIX 3B*).

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.*

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 (see Table A.2A.1)
Add H_2O to 1 L
Filter sterilize

Sodium acetate buffer, 0.1 M

Solution A: 11.55 mL glacial acetic acid per L (0.2 M) in water.

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

Referring to Table A.2A.3 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.3, prepare closest higher pH, then titrate with solution A.

Sodium phosphate buffer, 0.1 M

Solution A: 27.6 g $NaH_2PO_4 \cdot H_2O$ per L (0.2 M final) in water.

Solution B: 53.65 g $Na_2HPO_4 \cdot 7H_2O$ per L (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 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.4, prepare closest higher pH, then titrate with solution A.

SSC (sodium chloride/sodium citrate), 20×

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

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

800 mL H_2O
175 g NaCl (3 M)
7.4 g EDTA (20 mM)
24.0 g NaH_2PO_4 (0.20 mM)
Adjust pH with 10 N NaOH (~6.5 mL for pH 7.4)
Adjust volume to 1 L with H_2O

TAE (Tris/acetate/EDTA) electrophoresis buffer, 10×

24.2 Tris base
5.71 mL glacial acetic acid
3.72 g $Na_2EDTA \cdot 2H_2O$
H_2O to 1 L

TBE (Tris/borate/EDTA) electrophoresis buffer, 10×

108 g Tris base (890 mM)
55 g boric acid (890 mM)
960 mL H_2O
40 mL 0.5 M EDTA, pH 8.0 (20 mM final; see recipe above)

10× and 5× TBE tend to precipitate over time. If convenient, dilute to 2× or 1× immediately, or stir continuously.

TBS (Tris-buffered saline)

800 mL H_2O
8.00 g NaCl (0.137 M)
0.2 g KCl (2.7 mM)
3.0 g Tris base (24.8 mM)
Adjust pH as desired (usually to pH 8) with 1 M HCl
Adjust volume to 1 L with H_2O

Prepackaged TBS (pH 8.0), which is reconstituted by adding water, is commercially available from Sigma. This is very convenient if used in large quantities.

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 below)
1 mM EDTA, pH 8.0 (see recipe above)

Tris·Cl, 1 M

Dissolve 121 g Tris base in 800 mL H_2O
Adjust to desired pH with concentrated HCl
Adjust volume to 1 L 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 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.*

Always use high-quality Tris (lower-quality Tris can be recognized by its yellow appearance when dissolved).

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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