LABORATORY STOCK SOLUTIONS AND EQUIPMENT

APPENDIX 2

Common Stock Solutions, Buffers, and Media

APPENDIX 2A

RECIPES

This section describes the preparation of buffers and reagents used in this manual for cell culture, manipulation of tissue, and cell biological methods. When preparing solutions, use deionized or 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.

Acid, concentrated stock solutions

See Table A.2A.1.

Acid precipitation solution

1 M HCl
0.1 M sodium pyrophosphate

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

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

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

Acid/base	Molecular weight	% by weight	Molarity (approx.)	Specific gravity	1 M solution (ml/liter)
Acetic acid (glacial)	60.05	99.6	17.4	1.05	57.5
Ammonium hydroxide	35.0	28	14.8	0.90	67.6
Formic acid	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	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.

Current Protocols in Cell Biology (1998) A.2A.1-A.2A.10

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.

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.*

CMF-DPBS (calcium- and magnesium-free Dulbecco's phosphate-buffered saline)

8.00 g NaCl (0.137 M)
0.20 g KCl (2.7 mM)
2.16 g $Na_2HPO_4 \cdot 7H_2O$ (8.1 mM)
0.20 g KH_2PO_4 (1.1 mM)
H_2O to 1 liter
Store at room temperature

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.

DMEM (Dulbecco's modified Eagle medium), supplemented

Dulbecco's modified Eagle medium, high-glucose formulation (see APPENDIX 2B; e.g., Life Technologies), containing:
5%, 10%, or 20% (v/v) FBS, heat inactivated (optional; see recipe below)
1% (v/v) nonessential amino acids
2 mM L-glutamine

continued

100 U/ml penicillin
100 µg/ml streptomycin sulfate
Filter sterilize if anything nonsterile has been added
Store up to 1 month at 4°C

DMEM containing this set of additives is sometimes called "complete DMEM." The percentage of serum used is indicated after the medium name—e.g., "DMEM/5% FBS." Absence of a number indicates no serum is used. DMEM is also known as Dulbecco's minimum essential medium.

Ham's F-12 nutrient mixture (APPENDIX 2B; available commercially, e.g., from Life Technologies), is sometimes added to DMEM; the resulting medium is known as DMEM/F-12.

Because of the higher bicarbonate content, DMEM requires ~10% CO_2 to maintain pH 7.4.

Culture media containing glutamine and penicillin should be warmed to 37°C as few times as possible since components, especially glutamine, degrade rapidly at 37°C.

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

DTT (dithiothreitol), 1 M

Dissolve 1.55 g DTT in 10 ml water and 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.

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. To heat inactivate FBS, heat serum 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.

Repeated thawing and refreezing should be avoided, as it may cause denaturation of the serum.

Heat-inactivated FBS (FBS that has been treated with heat to inactivate complement protein and thus prevent an immunological reaction against cultured cells) is useful for a variety of purposes. It can be purchased commercially or made in the lab as described above.

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)
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 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 in the following order:
913.8 ml H_2O
86.2 ml concentrated HCl

HeBS (HEPES-buffered saline) solution, 2×

16.4 g NaCl
11.9 g HEPES acid
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

LB medium

Per liter:
10 g tryptone
5 g yeast extract
5 g NaCl
1 ml 1 M NaOH
Autoclave 25 min

Although the pH is adjusted to near 7 with NaOH, the medium is not very highly buffered, and the pH of a culture growing in the medium drops as the culture nears saturation.

The medium may also contain antibiotics (e.g., 50 µg/ml ampicillin, 12 µg/ml tetracycline), galactosides (e.g., 20 µg/ml Xgal, 0.1 mM IPTG), or other nutritional supplements added after the medium has been autoclaved.

To make LB agar for LB plates, add 5 g/liter agar or agarose.

$MgCl_2$, 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

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

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 1 liter

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 (see APPENDIX 3).

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 PMSF addition because PMSF has a tendency to form an insoluble precipitate in aqueous solution.

Polylysine-coated tissue culture surfaces

Prepare a stock solution by dissolving 100 mg polylysine in 100 ml water (poly-L-lysine or poly-D-lysine can be used; check specific protocol for choice of isomer) and filter sterilize through a 0.22-μm filter. Store in 5-ml aliquots at −20°C. When ready to use, dilute 1 part stock solution with 19 parts water to prepare a 50 μg/ml working solution.

To coat culture dishes, multiwell plates, or chamber slides: Fill tissue culture dishes, multiwell plates, or slide wells with the working solution and incubate 1 hr in a 37°C incubator, then remove solution by vacuum aspiration and allow surface to dry.

To coat coverslips: Sterilize coverslips by autoclaving or by incubating them in 95% ethanol and drying before coating. Place coverslips in a single layer in a petri dish containing working solution and incubate 1 hr at 37°C. Remove coverslips using sterile forceps and allow surface to dry.

Store coated tissue culture ware up to 3 months at 4°C. Use diluted solutions only once.

Potassium acetate buffer, 0.1 M

Solution A: 11.55 ml glacial acetic acid per liter (0.2 M) in water.

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

continued

Table A.2A.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).

Referring to Table A.2A.2 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.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 final) in water.

Solution B: 34.8 g K_2HPO_4 per liter (0.2 M final) 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 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.3, prepare closest higher pH, then titrate with solution A.

Saponin, 10% (w/v)

Dissolve 1 g saponin in 10 ml PBS (see recipe above)
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 SDS (sodium dodecyl sulfate or sodium lauryl sulfate) in H_2O 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.

Table A.2A.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).

SDS electrophoresis buffer, 5×

15.1 g Tris base
72.0 g glycine
5.0 g SDS
Distilled, deionized H_2O to 1 liter
Store up to 1 month at 0° to 4°C
Dilute to 1× before use

Do not adjust the pH of the stock solution; the pH is 8.3 when diluted to 1×.

Use purified SDS if appropriate.

SDS sample buffer

See Table A.2A.4.

Table A.2A.4 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[b,c]	400 µl	800 µl	~300 mM
H_2O	to 10 ml	to 10 ml	—

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

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 liter
Filter sterilize

Sodium acetate buffer, 0.1 M

Solution A: 11.55 ml glacial acetic acid 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.2 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.2, 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 liter (0.2 M final) in water.

Solution B: 53.65 g $Na_2HPO_4 \cdot 7H_2O$ per liter (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 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.3, prepare closest higher pH, then titrate with solution A.

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 liter

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)

TBS (Tris-buffered saline)

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

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)

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 below).

TEN (Tris/EDTA/NaCl) solution

40 mM Tris·Cl, pH 7.5 (see recipe below)
1 mM EDTA, pH 8.0 (see recipe above)
150 mM NaCl

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 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 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.*

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 above). Store up to several months at 4°C.

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.

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 above). DEPC inactivates ribonucleases by covalent modification (however, it cannot be used with Tris solutions because Tris inactivates DEPC).

   CAUTION: *DEPC is hazardous and a suspected carcinogen; follow appropriate precautions for handling, storage, and disposal.*

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 disposable gloves.

LITERATURE CITED

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