COMMONLY USED REAGENTS AND EQUIPMENT

BUFFERS AND STOCK SOLUTIONS

This collection describes the preparation of buffers and reagents used in the manipulation of nucleic acids and proteins (see Table A.2.1). When preparing solutions, use deionized, distilled water and reagents of the highest grade available. Sterilization—by filtration through a 0.22-µm filter or by autoclaving—is recommended for most applications. Recipes for the following can be found elsewhere in the manual: culture media (*UNIT 1.1*), antibiotics (Table 1.4.1), lactose analogs (Table 1.4.2), and enzyme buffers (*UNIT 3.4*).

CAUTION: *Handle strong acids and bases carefully.*

Table A.2.1 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

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 efficient.

Ammonium acetate, 10 M

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

BBS (BES-buffered solution), 2×

50 mM *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES; Calbiochem)
280 mM NaCl
1.5 mM Na_2HPO_4, pH 6.95
800 ml H_2O
Adjust pH to 6.95 with room temperature 1 M NaOH
H_2O to 1 liter

continued

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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.

$CaCl_2$, 1 M

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

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

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

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.*

HBSS (Hanks balanced salt solution)

5.4 mM KCl
0.3 mM Na_2HPO_4
0.4 mM KH_2PO_4
4.2 mM $NaHCO_3$
1.3 mM $CaCl_2$
0.5 mM $MgCl_2$
0.6 mM $MgSO_4$
137 mM NaCl
5.6 mM D-glucose
0.02% phenol red (optional)
Add H_2O to l liter and adjust pH to 7.4

HBSS can be purchased from Biofluids or Whittaker.

HBSS may be made or purchased without $CaCl_2$ and $MgCl_2$. These are optional components that usually have no effect on an experiment. In some cases, however, their presence may be detrimental to a procedure. Consult the individual protocol to see if the presence or absence of these components is recommended in the materials list.

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
Test for transfection efficiency and store at −20°C in 50-ml aliquots

An exact pH is extremely important for efficient transfection. The optimal pH range is 7.05 to 7.12.

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

MOPS buffer

0.2 M MOPS [3-(*N*-morpholino)propanesulfonic acid], pH 7.0
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

PBS (phosphate-buffered saline)

10× stock solution, 1 liter:	*Working solution, pH ~7.3:*
80 g NaCl	137 mM NaCl
2 g KCl	2.7 mM KCl
11.5 g $Na_2HPO_4 \cdot 7H_2O$	4.3 mM $Na_2HPO_4 \cdot 7H_2O$
2 g KH_2PO_4	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.2.2 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.2.2, prepare closest higher pH, then titrate with solution A.

Table A.2.2 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).

Potassium phosphate buffer, 0.1 M

Solution A: 27.2 g KH_2PO_4 per liter (0.2 M).

Solution B: 34.8 g K_2HPO_4 per liter (0.2 M).

Referring to Table A.2.3 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.

Table A.2.3 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
H_2O to 1000 ml
Dilute to 1× or 2× for working solution, as appropriate
Store up to 1 month at 0° to 4°C

Do not adjust the pH of the stock solution, as the solution is pH 8.3 when diluted.

SED (standard enzyme diluent)

20 mM Tris·Cl, pH 7.5
500 µg/ml bovine serum albumin (Pentax Fraction V)
10 mM 2-mercaptoethanol
Store up to 1 month at 4°C

Sodium acetate, 3 M

Dissolve 408 g sodium acetate·$3H_2O$ 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 ($NaC_2H_3O_2·3H_2O$)/liter (0.2 M).

Referring to Table A.2.2 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 $NaH_2PO_4·H_2O$ per liter (0.2 M).
Solution B: 53.65 g $Na_2HPO_4·7H_2O$ per liter (0.2 M).

Referring to Table A.2.3 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·$2H_2O$ (88 g/liter)
Adjust pH to 7.0 with 1 M HCl

STE buffer

10 mM Tris·Cl, pH 7.5
10 mM NaCl
1 mM EDTA, pH 8.0

TAE (Tris/acetate/EDTA) electrophoresis buffer

50× stock solution:
242 g Tris base
57.1 ml glacial acetic acid
37.2 g $Na_2EDTA·2H_2O$
H_2O to 1 liter

Working solution, pH ~8.5:
40 mM Tris·acetate
2 mM $Na_2EDTA·2H_2O$

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 (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

TEA (triethanolamine) solution

50 mM triethanolamine, pH ~11.5
0.1% Triton X-100
0.15 M NaCl

Add Triton X-100 as a 10% stock sterilized by Millipore filtration and stored in the dark to prevent photooxidation (stock is stable 5 years at room temperature).

TEN (Tris/EDTA/NaCl) solution

40 mM Tris·Cl, pH 7.5
1 mM EDTA, pH 8.0
150 mM NaCl
Store up to 6 months at room temperature

TM buffer, 10×

100 mM Tris·Cl, pH 8.0
100 mM $MgCl_2$

Tris-buffered saline (TBS)

100 mM Tris·Cl, pH 7.5
0.9% (150 mM) NaCl
Store up to several months at 4°C

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

Approximately 70 ml of HCl is needed to achieve a pH 7.4 solution, and approximately 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.*

TTBS (Tween 20/TBS)

0.1% Tween 20 in Tris-buffered saline (TBS; see recipe)
Store up to several months at 4°C

EQUIPMENT

Special equipment is also itemized in the materials list of each protocol. We have not attempted to list all items required for each procedure, but rather have noted those items that might not be readily available in the laboratory or that require special preparation. Listed below are standard pieces of equipment in the modern molecular biology laboratory, i.e., items used extensively in this manual and thus not included in the individual materials lists.

Autoclave

Balances, analytical and preparative

Bench protectors, plastic-backed (including "blue pads")

Centrifuges a low-speed (20,000 rpm) refrigerated centrifuge and an ultracentrifuge (20,000 to 80,000 rpm) are required for many procedures. Vertical ultracentrifuge rotors are very convenient for preparing plasmid DNA. At least one microcentrifuge that holds stan-

dard 1.5-ml microcentrifuge tubes is essential. It is also useful to have a large-capacity, low-speed centrifuge (such as the Beckman J-6M) for spinning down large bacterial cultures and a tabletop swinging-bucket centrifuge with adapters for spinning 96-well microtiter plates.

NOTE: *Centrifuge speeds are provided as* × g or as rpm (with example rotor models) throughout the manual. Readers should consult the nomograms in *APPENDIX 1G* to convert these speeds to their own rotor models.

Computer (PC or Macintosh) and printer

Darkroom and developing tanks or X- Omat automatic X-ray film developer.

Dry ice

Filtration apparatus for collecting acid precipitates on nitrocellulose filters or membrane.

Flasks, glass (e.g., Erlenmeyer, Florence)

Fraction collector

Freezers and refrigerators for 4°, –20°, and –70°C incubation and storage.

Fume hood

Geiger counter

Gel dryer

Gel electrophoresis equipment at least one full-size horizontal apparatus and one horizontal minigel apparatus, two sequencing gel setups for each person engaged in large-scale sequencing projects, one vertical gel apparatus for polyacrylamide protein gels, and specialized equipment for two-dimensional protein gels as required.

Heating blocks thermostat-controlled metal heating blocks that hold test tubes and/or microcentrifuge tubes are very convenient for carrying out enzymatic reactions.

Ice maker

Incubator (37°C) for growing bacteria. We recommend an incubator large enough to hold a "tissue culture" roller drum that can be used to grow 5-ml cultures in standard 18 × 150 mm test tubes. A convenient and durable tube roller is made by New Brunswick Scientific.

Incubator/shaker(s) an enclosed shaker (such as the New Brunswick Controlled Environment Incubator Shaker) that can spin 4-liter flasks is essential for growing 1-liter *E. coli* cultures. A rotary shaking water bath (New Brunswick R76) is useful for growing smaller cultures in flasks.

Light box for viewing autoradiograms.

Liquid nitrogen

Magnetic stirrers (with heater is useful).

Microcentrifuge, Eppendorf-type, maximum speed 12,000 to 14,000 rpm

Microcentrifuge tubes, 1.5-ml

Microwave oven to melt agar and agarose.

Mortar and pestle

Paper cutter large size, for 46 cm × 57 cm Whatman sheets.

Paper towels

Parafilm

Pasteur pipets and bulbs

PCR machine (Perkin-Elmer or MJ Research)

pH meter

pH paper

Pipettors that use disposable tips and dispense 1 to 1000 µl. It is best to have a set for each full-time researcher.

Plastic wrap (e.g., Saran Wrap)

Polaroid camera and UV transilluminator for taking photographs of stained gels.

Policemen, rubber or plastic

Power supplies 300-volt power supplies are sufficient for agarose gels; 2000-volt power supply required for DNA sequencing.

Racks, test tube

Radiation shield (Lucite or Plexiglas)

Radioactive ink

Radioactive waste containers, for liquid and solid waste

Refrigerator, 4°C

Safety glasses

Scalpels and blades

Scintillation counter

Scissors

Seal-A-Meal bag sealer or equivalent

Shakers, orbital and platform, room temperature or 37°C

Spectrophotometer UV and visible

Speedvac evaporator (Savant)

Tissue culture equipment CO_2 incubator, phase contrast microscope, liquid nitrogen storage container, and laminar flow hood.

UV cross-linker (e.g., Stratalinker, Stratagene)

UV light sources, long- and short-wave

UV transilluminator

Vacuum desiccator/lyophilizer

Vacuum oven

Vortex mixers

Water baths at least two with 80°C capacity

Water purification equipment or glass distillation apparatus to purify all water used in molecular biology experiments.

X-ray film cassettes and intensifying screens

LITERATURE CITED

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

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