# LABORATORY STOCK SOLUTIONS AND EQUIPMENT



## Common Stock Solutions and Buffers

This section describes the preparation of buffers and reagents used in this manual. When preparing solutions, use Milli-Q-purified water (or equivalent) 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. Where storage conditions are not specified, store up to 6 months at room temperature. Discard any solution that shows evidence of contamination, precipitation, or discoloration.

Standardized and reference reagents are sometimes required—e.g., for quality control, positive and negative controls, equipment calibration, and assay validation. Environmental and chemical standards are available from a number of suppliers, including the National Institute of Standards and Technology (NIST), AccuStandard Inc., and Sigma Chemical Co. Consult the *LabGuide*, published annually by the American Chemical Society (ACS) for other suppliers (e.g., ACS, 1999).

***Acid, concentrated stock solutions***

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 hydroxide, concentrated stock solution***

See Table A.2A.1.

***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.

***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.

***$CaCl_2$, 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

***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 the disodium salt, is difficult to dissolve at this concentration unless the pH is increased to between 7 and 8.*

***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)

*continued*

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

***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

***NaCl (saline), 0.9% (w/v)***

9 g NaCl (154 mM final)
$H_2O$ to 1 liter

***NaOH, 10 M***

Dissolve 400 g NaOH in 450 ml $H_2O$
$H_2O$ 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

***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.

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.

*continued*

*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.*

**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).

**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).

**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.

***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.*

***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.

***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.*

***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

***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.*

## LITERATURE CITED

American Chemical Society (ACS). 1999. LabGuide 98-99. ACS, Washington, D.C. [also available online at *http://pubs.acs.org./labguide*].

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