Stock Solutions

This section of the manual contains instructions for preparing stock solutions that are commonly used in laboratory protocols for the analysis of proteins, RNA, and DNA, and the visualization of gene and/or protein expression. Additional information regarding specific applications and uses of these reagents can be found in the cross-references to Cold Spring Harbor Laboratory Press manuals (see key at the front of this book) that are designated following each recipe. Each of these stock solutions is provided as either the most convenient concentration or the most frequently used stock concentration. Each can be diluted to additional working stock concentrations to be used in the protocols of an individual laboratory.

All chemicals must be reagent grade or molecular biology grade, and the water used in the preparation of all solutions must be the highest quality available. Use sterile, glass-distilled deionized water whenever possible. Unless otherwise stated, most solutions require sterilization either by filtration or autoclaving.

Where the directions call for filter sterilization, solutions should be passed through a 0.22-μm filter prior to storage at the recommended temperature. There are a number of commercially available filters that are suitable for syringe, large-scale, or bottle-top filtration. Where autoclaving is recommended, follow the directions in the table below. Most solutions can be stored at room temperature for at least 6 months, unless otherwise specified. Be sure that repeated use of a common stock of these solutions does not result in contamination. Aliquots will help avoid this problem.

Caution: See Appendix 1 for appropriate handling of materials marked with ☣.

10 M Ammonium Acetate ($NH_4C_2H_3O_2$) GA MC2

Dissolve 771 g of ammonium acetate▼ (m.w. = 77.1 g/mole) in 800 ml of H_2O. Make up to a final volume of 1 liter with H_2O, sterilize by filtration, and store at room temperature.

1.43 M Ammonium Chloride (NH_4Cl) (Used as 100× Ammonium) PB

Dissolve 11.5 g of NH_4Cl▼ (m.w. = 53.5 g/mole) in 100 ml of ultrapure H_2O. Adjust to 150 ml, sterilize by filtration, and store at room temperature.

1 M Calcium Chloride ($CaCl_2$) MC2 C

Dissolve 44 g of $CaCl_2$▼ •$6H_2O$ in 200 ml of pure H_2O (Milli-Q or equivalent). Sterilize the solution by passage through a 0.22-µm filter. Store in 1-ml aliquots at –20°C.

> ***Note:*** When preparing competent cells, thaw an aliquot of 1 M $CaCl_2$ and dilute it to 100 ml with pure H_2O. Sterilize the solution by filtration through a filter (0.45-µm pore size), and then chill it to 0°C.

1 M Dithiothreitol (DTT) DNAS SPP AB MC2

Dissolve 1.5 g of DTT▼ (DL-dithiothreitol, anhydrous m.w.=154.25) in 8 ml of deionized or distilled H_2O. Adjust volume to 10 ml, dispense into 1-ml aliquots, and store in the dark (wrapped in aluminum foil) at –20°C (indefinitely).

> ***Note:*** Do not autoclave DTT or solutions containing it.

10 mg/ml DNA (Salmon Sperm) GA MC2 C

Sonicated, denatured salmon sperm DNA is commercially available at a concentration of 10 mg/ml but is fairly expensive. A large economical supply of salmon sperm DNA stock solution can be prepared in the laboratory, although the process is lengthy.

Large-scale Preparation

To prepare a stock solution, dissolve 1 g of desiccated salmon sperm DNA in 100 ml of H_2O by stirring for at least 1 day. Add NaCl to a final concentration of 100 mM and extract with TE-saturated phenol ☤ (pH 8.0) (1:1). Shear the extracted DNA by sonication or by repeatedly passing (10–20 times) the DNA through a 16–18-gauge needle. Analyze on an agarose gel along with the appropriate molecular-weight markers to determine the approximate size.

Precipitate the DNA with ethanol ☤ according to standard protocols and dissolve in H_2O to achieve a final concentration of 10 mg/ml. Divide into aliquots (e.g., 10 ml) and place in a boiling-water bath for 10 minutes to denature the DNA. Rapidly chill the denatured DNA on ice. Store at –20°C.

> ***Note:*** For use in hybridizations, the desired size range is approximately 500–1000 bp. For lithium acetate transformations of yeast, much larger salmon sperm DNA (5–10 kb) is preferred for use as a carrier.

Small-scale Preparation

Dissolve 10 mg of salmon sperm DNA in 1 ml of sterile H_2O in a polycarbonate tube. Sonicate five times, 30 seconds each time, at maximum power, chilling tube on ice between bursts. Check molecular size of DNA by gel electrophoresis; it should be 200–400 bp. Store in 50-µl aliquots for up to 1 year at –20°C.

DNA, Sonicated Herring Sperm (2 mg/ml) C

Resuspend 1 g of herring sperm in a convenient volume (50 ml of H_2O) by sonicating briefly. The DNA is now ready to be sheared into short molecules by sonication. Place tube containing herring sperm DNA solution in an ice bath (the tube must be stable even if ice begins to melt). Place the sonicator probe in the DNA solution (without touching bottom of vessel). Turn on the sonicator to 50% power for 10 minutes or until there is a uniform and obvious decrease in viscosity. Do not allow the tube containing the DNA to become hot to the touch. After sonication, dilute the DNA with H_2O to a final concentration of 2 mg/ml, freeze in 50-ml aliquots, and thaw as needed.

> ***Notes:*** Some investigators recommend that salmon sperm DNA be boiled and chilled before each use. In general, this solution is not sterilized.
>
> Use only buffer-saturated phenol ☤.

70% (v/v) Ethanol (EtOH) GA

To prepare a solution of approximately 70% (v/v), mix 70 ml of absolute ethanol▼ with 30 ml of sterile H_2O. Do not autoclave. Prepare as needed or store at –20°C. Sterilization is not required.

Ethidium Bromide (EtBr) (10 mg/ml) MC2 C GA

Add 1 g of ethidium bromide▼ to 100 ml of H_2O. Stir on a magnetic stirrer for several hours to ensure that the dye has dissolved. Wrap the container in aluminum foil or transfer the solution to a dark bottle and store at room temperature.

0.5 M EDTA (Disodium Ethylene Diamine Tetra-Acetate, pH 8.0) MC2 AB

EDTA and EGTA are used to chelate divalent cations. EDTA chelates both Mg^{++} and Ca^{++}; EGTA can be used to chelate Ca^{++} in the presence of Mg^{++}. EDTA has a molecular weight of 292.2 as the free acid and 372.3 as the *di*-Na salt (dihydrate). $Na_2EDTA{\cdot}2H_2O$ is only soluble at basic pH.

To make a 500 mM solution, add 181.6 g of $Na_2EDTA{\cdot}2H_2O$ to about 800 ml of distilled H_2O and, while stirring with a magnetic stirrer, adjust to pH 8.0 with pellets of NaOH▼ (~20 g of NaOH is required). If necessary, make up to 1 liter volume with distilled H_2O. Sterilize by autoclaving. Store at room temperature.

> ***Note:*** The disodium salt of EDTA will not go into solution until the pH of the solution is adjusted to approximately 8.0 by the addition of NaOH.

1 M EGTA (Ethylene Glycol-Bis[β-Aminoether]N,N,′N′,N -Tetra-Acetic Acid) PB

EGTA has a molecular weight of 380.4. Add 250 g and 53g of NaOH▼ pellets to 400 ml of distilled H_2O. Adjust pH to 7.0 with HCl▼. Adjust to 660 ml with H_2O. Filter. Autoclave. Store at 4°C (will last ~1 month). Use 1:100 in liquid and solid media (final 10 mM).

For a 100 mM EGTA stock solution, add 3.8 g to about 20 ml of distilled H_2O and bring to pH 11 with NaOH; then bring to pH 8.0 with HCl and add H_2O to a final volume of 100 ml.

50× Glucose (150-ml Stock Solution) PB

D-Glucose	2 M	54 g

Dissolve and adjust to 150 ml with ultrapure H_2O. Filter-sterilize and store at room temperature.

Isopropyl-β-D-Thiogalactopyranoside (IPTG) MC2 GA

Dissolve 2 g of IPTG▼ (m.w. = 238.3 g/mole) in 8 ml of distilled H_2O. Adjust the volume of the solution to 10 ml with distilled H_2O and sterilize by filtration through a 0.22-µm disposable filter. Dispense the solution into 1-ml aliquots and store them at –20°C.

10 M Lithium Chloride (LiCl) DROS

LiCl▼ has a molecular weight of 42.40 (anhydrous) and 60.41 (1-hydrate) and is deliquescent. For a 10 M LiCl solution, dissolve 424 g of anhydrous LiCl in 1 liter of distilled H_2O. Autoclave. Store at room temperature.

1 M Manganese Chloride ($MnCl_2$) DROS

The molecular weight of $MnCl_2$▼ •$4H_2O$ is 197.0. Prepare as a 1 M stock solution by dissolving 197 g in 1 liter of distilled H_2O. Autoclave. Store at room temperature.

1 M Magnesium Acetate MC2

Dissolve 214.46 g of magnesium acetate▼ •$4H_2O$ in 800 ml of H_2O. Adjust the volume to 1 liter with H_2O. Sterilize by filtration. Store at room temperature.

1 M Magnesium Chloride ($MgCl_2$) MC2 C GA

Dissolve 203.3 g of $MgCl_2$▼ •$6H_2O$ in 800 ml of H_2O. Adjust the volume to 1 liter with H_2O. Dispense into aliquots and sterilize by autoclaving.

> ***Note:*** $MgCl_2$ is extremely hygroscopic. Buy small bottles (e.g., 100 g) and do not store opened bottles for long periods of time. Once the crystals become saturated with H_2O, dispose of the chemical properly.

1 M Magnesium Sulfate ($MgSO_4$) DROS PB

To make a 1 M solution, dissolve 120 g of $MgSO_4$▼ (anhydrous) or 246 g of $MgSO_4$•$7H_2O$ to 1 liter of distilled H_2O. Sterilize by autoclaving. Store at room temperature.

Preparation of Phenol MC2 PB

Most batches of commercial liquified phenol▼ are clear and colorless and can be used in molecular cloning without redistillation. Occasionally, batches of liquified phenol are pink or yellow, and these should be rejected and returned to the manufacturer. Crystalline phenol is not recommended because it must be redistilled at 160°C to remove oxidation products, such as quinones, that cause the breakdown of phosphodiester bonds or cause cross-linking of RNA and DNA.

Equilibration of Phenol

Before use, phenol must be equilibrated to a pH >7.8 because DNA will partition into the organic phase at acid pH.

1. Liquified phenol should be stored at –20°C. As needed, remove the phenol from the freezer, allow it to warm to room temperature, and then melt it at 68°C. Add hydroxyquinoline to a final concentration of 0.1%. This compound is an antioxidant, a partial inhibitor of RNase, and a weak chelator of metal ions. In addition, its yellow color provides a convenient way to identify the organic phase.
2. To the melted phenol, add an equal volume of buffer (usually 0.5 M Tris-HCl [pH 8.0] at room temperature). Stir the mixture on a magnetic stirrer for 15 minutes, and then turn off the stirrer. When the two phases have separated, aspirate as much as possible of the upper (aqueous) phase using a glass pipette attached to a vacuum line equipped with traps.
3. Add an equal volume of 0.1 M Tris-HCl (pH 8.0) to the phenol. Stir the mixture on a magnetic stirrer for 15 minutes, and then turn off the stirrer. Remove the upper aqueous phase as described in step 2. Repeat the extractions until the pH of the phenolic phase is >7.8 (as measured with pH paper).
4. After the phenol is equilibrated and the final aqueous phase has been removed, add 0.1 volume of 0.1 M Tris-HCl (pH 8.0) containing 0.2% β-mercaptoethanol▼. The phenol solution may be stored in this form under 100 mM Tris-HCl (pH 8.0) in a light-tight bottle at 4°C for periods of up to 1 month.

Phenol:Chloroform MC2

Mix equal amounts of phenol▼ and chloroform▼. Equilibrate the mixture by extracting several times with 0.1 M Tris-HCl (pH 7.6). Store the equilibrat-

ed mixture under an equal volume of 0.01 M Tris-HCl (pH 7.6) at 4°C in dark glass bottles.

0.2 M PIPES (Piperazine-N′N-bis [2-Ethanesulfonic Acid]; 1,4-Piperazinediethanesulfonic acid) MC2

Dissolve 64.87 g of PIPES monosodium salt (m.w. 324.3) into 1 liter of distilled H_2O. Filter to sterilize.

Polyethylene Glycol (PEG)

PEG▼ is available in a range of molecular weights from about 200 to 8000 and is used as a protective agent for causing cell fusion and (as a high-molecular-weight wax) for embedding.

50% PEG 3350 FY

50% PEG▼ 3350 in 0.1 M lithium acetate (pH 4.9). Prepare a 50% solution by dissolving 50 g of PEG 3350 in 50 ml of 0.2 M lithium acetate (pH 4.9). Make up to 100 µl with H_2O.

20% PEG 8000/2.5 M NaCl GA

Add 20 g of the PEG▼ 8000 to a beaker containing 50 ml of 5 M NaCl (m.w. = 58.44 g/mole) and sufficient H_2O to make a final volume of 100 ml. Stir with a magnetic stirring bar.

8 M Potassium Acetate (KOAc) DNAS GA

Dissolve 78.5 g of potassium acetate (m.w. = 98.14) in 80 ml of deionized H_2O. Add deionized or distilled H_2O to make a total volume of 100 ml of solution. Filter-sterilize through a 0.22-µm filter. Store at room temperature (indefinitely).

1 M Potassium Acetate (KOAc, pH 7.5) MC2

Dissolve 9.82 g of potassium acetate in 90 ml of pure H_2O (Milli-Q or equivalent). Adjust the pH to 7.5 with 2 M acetic acid. Add pure H_2O to 100 ml. Divide the solution into aliquots and store them at –20°C.

88 mM Potassium Phosphate (Used as 100× Phosphate) PB

Dissolve 2.3 g of K_2HPO_4 ▼ in 150 ml of ultrapure H_2O. Filter-sterilize and store at room temperature.

Potassium Chloride (KCl) GA

For a 1 M solution of KCl ▼ (m.w. = 74.55), dissolve 74.55 g of KCl in 900 ml of distilled H_2O, and make up to 1 liter. Sterilize by autoclaving.

10 N Sodium Hydroxide (NaOH) MC2 C GA

The preparation of a concentrated NaOH ▼ (10 N) solution entails an exothermic reaction. Extreme caution must be taken to avoid chemical burns and breakage of glass containers. If possible, use heavy plastic beakers.

To prepare 10 N NaOH, add 400 g of NaOH pellets (m.w. = 40) to a beaker containing approximately 0.9 liter of H_2O that is being stirred with a magnetic stirring bar. *Do not add H_2O to the NaOH pellets.* The beaker can be placed in a container of ice. After the pellets have completely dissolved, adjust the final volume to 1 liter with H_2O. Sterilization is not required.

Alternatively, to avoid the use of NaOH pellets in preparing 10 N NaOH, use the commercially available concentrated NaOH solution. Add 524 ml of 50% NaOH solution (19.1 N) to 476 ml of H_2O while stirring with a magnetic stirring bar.

10% Sodium Azide C AB

Dissolve 10 g of sodium azide ▼ in 100 ml of distilled H_2O. Store at room temperature.

1 M Sodium Azide (Na Azide) C AB DROS

For a 1 M solution, dissolve 6.5 g of Na azide ▼ (m.w. 65.02) in 100 ml of distilled H_2O. Na azide is often added to buffers and other solutions to inhibit the growth of bacteria and molds.

3 M Sodium Acetate (pH 5.2 and pH 7.0) MC2 C GA

Dissolve 408.1 g of sodium acetate ▼ $\cdot 3H_2O$ (m.w. 136) in 800 ml of H_2O. Adjust the pH to 5.2 with glacial acetic acid or adjust the pH to 7.0 with dilute acetic acid. Adjust the volume to 1 liter with H_2O. Dispense into aliquots and sterilize by autoclaving. Store at room temperature.

1 M Sodium Bicarbonate ($NaHCO_3$) (Used as 50× Bicarbonate) PB

Dissolve 12.6 g of $NaHCO_3$ (m.w. 84) in 100 ml of ultrapure H_2O. Adjust to 150 ml with ultrapure H_2O to yield a 1 M solution. Filter-sterilize and store at room temperature.

5 M Sodium Chloride (NaCl) MC2 C GA

Dissolve 292.2 g of NaCl in 800 ml of H_2O. Adjust the volume to 1 liter with H_2O. Dispense into aliquots and sterilize by autoclaving. Store at room temperature.

10% Sodium Dodecyl Sulfate (SDS) DNAS

Dissolve 10 g of electrophoresis-grade SDS▼ (m.w. = 288.37) in 80 ml of deionized H_2O. Add deionized or distilled H_2O to make a total volume of 100 ml of solution. Store at room temperature (indefinitely).

> ***Note:*** It is useful to add the SDS a few grams at a time in order to facilitate the preparation of this stock. SDS can also be made up in a 20% stock. Sterilize by autoclaving. SDS is the same as sodium lauryl sulfate.

100% (w/v) Trichloroacetic Acid (TCA) GA MC2 AB

The safest method for preparing a TCA stock solution to avoid weighing out the chemical is as follows: Add 100 ml of H_2O to a bottle containing 500 g of TCA▼. (This chemical is very soluble in H_2O.) Stir with a magnetic stirring bar until completely dissolved. Add more H_2O as needed. Adjust the final volume to 500 ml with H_2O. Store in a dark glass bottle in an acid-safe hood. Sterilization is not required.

TCA undergoes decomposition at concentrations below 30%. Dilutions should be prepared just before use.

1.5 M Tris (pH 8.8) MC2 GA C

Dissolve 181.5 g of Tris▼ base in 800 ml of distilled H_2O. Adjust the pH to 8.8 with concentrated HCl▼. Adjust the volume to 1 liter. Dispense in convenient volumes and sterilize by autoclaving. Store at room temperature.

1.0 M Tris (pH 6.8) MC2 GA C

Dissolve 12.1 g of Tris▼ base in 80 ml of distilled H_2O. Adjust the pH to 6.8 with concentrated HCl▼. Adjust the volume to 100 ml. Sterilize by autoclaving. Store at room temperature.

1 M Tris MC2

Dissolve 121.1 g of Tris▼ base in 800 ml of H_2O. Adjust the pH to the desired value by adding concentrated HCl▼.

pH	HCl
7.4	70 ml
7.6	60 ml
8.0	42 ml

Allow the solution to cool to room temperature before making final adjustments to the pH. Adjust the volume of the solution to 1 liter with H_2O. Dispense into aliquots and sterilize by autoclaving. Store at room temperature.

> ***Note:*** If the 1 M solution has a yellow color, discard it and obtain better quality Tris. Although many types of electrodes do not accurately measure the pH of Tris solutions, suitable electrodes can be obtained from most manufacturers. The pH of Tris solutions is temperature-dependent and decreases approximately 0.03 pH units for each 1°C increase in temperature. For example, a 0.05 M solution has pH values of 9.5, 8.9, and 8.6 at 5°C, 25°C, and 37°C, respectively.

Concentrations of Commercial Liquids AB

Compound	Molecular weight	Molarity	pH of dilute solutions		
			1 M	0.1 M	0.01 M
Acetic acid▼, glacial	60.05	17.4	2.4	2.9	3.4
Formic acid▼	46.02	23.4			
Hydrochloric acid▼, 38%	36.47	11.6	0.1	1.1	2.02
Nitric acid▼, 70%	63.02	16			
Phosphoric acid▼	98.0	18.1	1.5		
Sulfuric acid▼	98.08	18			
Ammonium hydroxide▼	35.0	14.8			
Ethanolamine▼, 99%	61.08	16.5			
Triethylamine▼	101.19	7.16			
Formaldehyde▼, 37%	30.3	12.2			
Hydrogen peroxide▼, 30%	34.02	8.8			
β-Mercaptoethanol▼	78.13	14.4			

Autoclaving Conditions MP

Material	Sterilizing cycle	Drying cycle
Surgical instruments	270°F (132°C), 30 minutes	270°F, 30 minutes
Glassware ▼	270°F, 30 minutes	270°F, 30 minutes
Liquids	250°F (121°C), 30 minutes	none
Small polypropylene tubes and caps[a]	250–270°F, 30 minutes	250–270°F, 30 minutes
Plastic pipette tips[a]	250–270°F, 30 minutes	250–270°F, 30 minutes

[a]Check with the manufacturer for the precise autoclaving temperature.