APPENDIX 1

Common Reagents

COMPILED BY JOAN KOBORI

All chemicals must be reagent grade or molecular biology grade. All H_2O used in the preparation of solutions must be of the highest quality available in the standard molecular biology laboratory. Use sterile, glass-distilled, deionized H_2O (purified through a Milli-Q filter or similar type of system) whenever possible. Unless otherwise stated, most prepared solutions require sterilization either by filtration through a 0.22-μm filter or by autoclaving at 15 psi on liquid cycle at 121°C for 20–30 minutes. Use autoclaved H_2O and sterile measuring devices for the preparation and use of solutions from sterile stock solutions and reagents. These measures ensure that the storage life of the reagents is as long as possible. In general, solutions prepared from dry chemicals and sterile H_2O do not need additional sterilization. For some solutions, sterilization is not required (e.g., acids, bases, and some organic compounds) because microbial growth cannot occur.

Unless specifically stated otherwise, all stock solutions and buffers can be stored at room temperature for at least 6 months. Divide large volumes into smaller aliquots for storage. Allow stock chemicals stored at 4°C or –20°C to reach room temperature before opening to prevent the accumulation of condensation within the reagent and therefore ensure more accurate measurement.

Solutions designated by percentage w/v are defined as the solute weight in grams per 100 ml and solutions designated by percentage v/v are defined as the constituent volume in milliliters per 100 ml of total volume. Buffer pH is given for a solution at 25°C. Information on molarities of standard concentrated acids and bases is also provided.

Important safety precautions for the reagents used here are provided in Appendix 3. In general, the use of protective clothing, gloves, and face protection are required when exposure to the skin can result in burns or absorption of a toxic chemical. Face masks (disposable covers for the mouth and nose) are needed when inhalation of a material is to be avoided.

STANDARD STOCK SOLUTIONS

10 M Ammonium Acetate

Dissolve 771 g of ammonium acetate (m.w. = 77.1 g/mole) in sufficient H_2O to make a final volume of 1 liter. Sterilize the solution by passing it through a 0.22-μm filter.

10 mg/ml BSA

Add 100 mg of BSA (Fraction V or molecular biology grade, DNase-free) to a 15-ml polypropylene tube containing 9.5 ml of H_2O. To reduce denaturation, always add protein to an aqueous solution instead of adding an aqueous solution to a protein. Gently rock the capped tube until the BSA is completely dissolved. Do not mix by vortexing (this causes foaming, which indicates protein denaturation). Adjust the final volume to 10 ml with H_2O. Divide into aliquots and store at –20°C. In general, this solution is not sterilized.

100x Denhardt's Reagent

Component and final concentration	Amount to add per 100 ml
2% Ficoll (Type 400)	2 g
2% polyvinylpyrrolidone (PVP-40)	2 g
2% BSA (Fraction V)	2 g
H_2O	to make 100 ml

Dissolve the components in the H_2O. Filter to sterilize and remove particulate matter. Divide into aliquots and store at –20°C.

10x Standard DNA Ligase Buffer

Different conditions for using bacteriophage T4 DNA ligase have been reported. A standard buffer for sticky-end and blunt-end ligation is given here. The optional spermidine is recommended for blunt-end ligations.

Component and final concentration	Amount to add per 10 ml
0.5 M Tris-Cl	5 ml of 1 M (pH 7.6 at 25°C)
100 mM $MgCl_2$	1 ml of 1 M
100 mM DTT	1 ml of 1 M
2 mM ATP	200 μl of 100 mM
5 mM spermidine HCl (*optional*)	50 μl of 1 M
0.5 mg/ml BSA (Fraction V) (*optional*)	0.5 ml of 10 mg/ml
H_2O	2.25 ml

Divide into small aliquots and store at −20°C. In general, this buffer is not sterilized.

100 mM dNTP Solutions

Stock solutions of purified dNTPs can be purchased as 100 mM solutions. These solutions can be stored at −80°C for at least 6 months.

To prepare 100 mM stock solutions, dissolve the appropriate amount of dNTP in H_2O, adjust the pH to approximately 7 with 1 M Tris base, and then determine the concentration precisely. These 100 mM stock preparations are typically found to be 85–95 mM. Therefore, adding less than the calculated volume of H_2O is recommended. In general, these stock solutions are not sterilized.

To determine the concentrations, serially dilute the 100 mM stock solutions with 10 mM Tris-Cl or phosphate buffer (pH 7.0) to approximately 10 µM. Adjust the spectrophotometer to zero with the dilution buffer. Use a quartz cuvette with a 1-cm path length and read the OD of each solution at the wavelength designated in the table below. Using the extinction coefficients (ε) listed in the table below, calculate the concentration of each dNTP solution as follows:

$$\text{molar concentration} = \frac{\text{measured OD} \times \text{dilution factor}}{\varepsilon}$$

dNTP	Wavelength (nm)	ε ($M^{-1}cm^{-1}$)
dATP	259	1.54×10^4
dCTP	271	9.10×10^3
dGTP	253	1.37×10^4
dTTP	260	7.40×10^3

Note: Many protocols call for mixtures of dNTPs at a set molarity (generally 0.5–10 mM). For example, a 10 mM dNTP mixture denotes a solution containing all four dNTPs, each at a final concentration of 10 mM, prepared by diluting concentrated dNTP stock solutions with H_2O as follows:

10 mM dNTP mixture

Component and final concentration	Amount to add per 20 µl
10 mM dATP	2 µl of 100 mM
10 mM dCTP	2 µl of 100 mM
10 mM dGTP	2 µl of 100 mM
10 mM dTTP	2 µl of 100 mM
H_2O	12 µl

Mix the components in a 1-ml microcentrifuge tube. This mixture can be stored at −20°C for at least 6 months.

1 M DTT

The simplest approach for preparing a 1 M stock solution is to add 32.4 ml of H_2O to a 5-g bottle of DTT. Divide into aliquots and store at –20°C. This procedure avoids the weighing of this foul-smelling chemical.

Alternatively, transfer 100 mg of DTT (m.w. = 154.25 g/mole) into a microcentrifuge tube and add 0.65 ml of H_2O to make a 1 M solution. Sterilization is not required.

0.5 M EDTA

The easiest method for preparing this common stock solution is to generate the trisodium salt of EDTA by preparing an equimolar solution of Na_2 EDTA and NaOH (i.e., each is 0.5 M). The trisodium salt is more soluble than the disodium salt. The pH of this solution should be approximately 8. This stock solution is adequate for all molecular biology protocols.

To prepare 0.5 M EDTA, combine 186.1 g of Na_2 EDTA · $2H_2O$ (m.w. = 372.2 g/mole), 20 g of **NaOH** (m.w. = 40 g/mole), and H_2O to make a final volume of 1 liter.

NaOH (see Appendix for Caution)

1 M HEPES

Dissolve 23.8 g of HEPES free acid (m.w. = 238.3 g/mole) in approximately 90 ml of H_2O. Adjust the pH with **NaOH** (the useful pH range is 6.8 to 8.2) and then adjust the final volume to 100 ml with H_2O.

NaOH (see Appendix for Caution)

1 N HCl

Standard concentrated HCl is 36% (w/w) or 11.6 N. To avoid severe burns caused by splattering, *always add acid to H_2O;* do not add H_2O to acid. Sterilization is not required.

For 100 ml of solution, add 8.6 ml of **concentrated HCl** to 91.4 ml of H_2O.

concentrated HCl (see Appendix for Caution)

25 mg/ml IPTG

Dissolve 250 mg of IPTG (m.w. = 238.3 g/mole) in 10 ml of H_2O. Divide into aliquots and store at –20°C. In general, this solution is not sterilized.

1 M $MgCl_2$

Dissolve 20.3 g of $MgCl_2$ · $6H_2O$ (m.w. = 203.3 g/mole) in sufficient H_2O to make a final volume of 100 ml.

20% PEG 8000/2.5 M NaCl

Component and final concentration	Amount to add per 100 ml
20% (w/v) PEG 8000	20 g
2.5 M NaCl	50 ml of 5 M NaCl or 14.6 g of solid NaCl
H_2O	to make 100 ml

Add the PEG 8000 to a beaker containing the 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.

100 mM PMSF

Dissolve 174 mg of **PMSF** (m.w. = 174.2 g/mole) in sufficient isopropanol to make a final volume of 10 ml. Divide into aliquots and store in foil-wrapped tubes at –20°C. Sterilization is not required.

PMSF (see Appendix for Caution)

8 M Potassium Acetate

Dissolve 78.5 g of potassium acetate (m.w. = 98.14 g/mole) in sufficient H_2O to make a final volume of 100 ml.

1 M KCl

Dissolve 7.46 g of KCl (m.w. = 74.55 g/mole) in sufficient H_2O to make a final volume of 100 ml.

20 mg/ml Proteinase K

Add 200 mg of proteinase K to a 15-ml polypropylene tube containing 9.5 ml of H_2O. To reduce denaturation, always add protein to an aqueous solution instead of adding an aqueous solution to a protein. Gently rock the capped tube until the proteinase K is completely dissolved. Do not mix by vortexing (this causes foaming, which indicates protein denaturation). Adjust the final volume to 10 ml. Divide into aliquots and store at –20°C. In general, this solution is not sterilized.

10 mg/ml RNase A (DNase-free)

To avoid contamination of RNase A, wear gloves and do not allow RNase A to come in contact with any laboratory surfaces or equipment used for RNA work. Dissolve 10 mg of pancreatic RNase A in 1 ml of 10 mM sodium acetate (pH 5.0). Place in a boiling-water bath for 15 minutes to inactivate any contaminating DNase. Adjust the pH to 7.5 with 1 M Tris-Cl. Store at –20°C. In general, this solution is not sterilized.

10 mg/ml Salmon Sperm DNA

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, but the process is lengthy.

To prepare the 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 **phenol** (see Appendix 2). 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. (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.) Precipitate the DNA with ethanol (see Appendix 2) and dissolve in H_2O at 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.

Some investigators recommend that salmon sperm DNA be boiled and chilled before each use. In general, this solution is not sterilized.

phenol (see Appendix for Caution)

3 M Sodium Acetate

Dissolve 40.8 g of sodium acetate trihydrate (m.w. = 136.1 g/mole) in approximately 90 ml of H_2O. Adjust the pH of the solution to 5.2 with **glacial acetic acid** and then adjust the final volume to 100 ml with H_2O.

glacial acetic acid (see Appendix for Caution)

5 M NaCl

Dissolve 29.2 g of NaCl (m.w. = 58.44 g/mole) in sufficient H_2O to make a final volume of 100 ml.

10 N NaOH

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 g/mole) 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.

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.

NaOH (see Appendix for Caution)

10% (w/v) SDS

Carefully weigh 100 g of **SDS** and slowly transfer it into a beaker containing approximately 0.9 liter of H_2O. Stir with a magnetic stirring bar until completely dissolved. Adjust the final volume to 1 liter. A 20% stock solution of SDS (200 g in 1 liter) can also be prepared if desired. Sterilization is not required.

SDS (see Appendix for Caution)

2 M Sorbitol

Dissolve 36.4 g of sorbitol (m.w. = 182.2 g/mole) in sufficient H_2O to make a final volume of 100 ml.

1 M Spermidine

Dissolve 2.55 g of spermidine trihydrochloride (m.w. = 254.6 g/mole) in sufficient H_2O to make a final volume of 10 ml. Divide into aliquots and store at –20°C. In general, this solution is not sterilized.

1 M Spermine

Dissolve 3.48 g of spermine tetrahydrochloride (m.w. = 348.2 g/mole) in sufficient H_2O to make a final volume of 10 ml. Divide into aliquots and store at –20°C. In general, this solution is not sterilized.

20x SSC

Component and final concentration	Amount to add per 1 liter
300 mM trisodium citrate (dihydrate)	88.2 g
3 M NaCl	175.3 g
H_2O	to make 1 liter

Dissolve the trisodium citrate dihydrate (m.w. = 294.1 g/mole) and the NaCl (m.w. = 58.44 g/mole) in approximately 0.9 liter of H_2O. Adjust the pH to 7.0 by adding a few drops of 10 N **NaOH**. Adjust the final volume to 1 liter with H_2O.

NaOH (see Appendix for Caution)

100% (w/v) TCA

The safest method for preparing a TCA stock solution is to avoid weighing out the chemical 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. Sterilization is not required.

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

TCA (see Appendix for Caution)

2.5% X-gal

Dissolve 25 mg of X-gal in 1 ml of **DMF**. Store in a foil-wrapped polypropylene tube at –20°C. Sterilization is not required.

DMF (see Appendix for Caution)

MOLARITIES OF CONCENTRATED ACIDS AND BASES

	Percentage solution (w/w)	Molarity (M)
Acids		
glacial acetic acid	99–100	17.4
formic acid	90	23.4
HCl	36	11.6
nitric acid	70	15.7
phosphoric acid	85	14.6
sulfuric acid	95	18
Bases		
ammonium hydroxide	28 (NH_3)	14.8
KOH	50	13.5
NaOH	50	19.1

COMMON LABORATORY SOLUTIONS

DEPC-treated H_2O

Add 100 µl of fresh **DEPC** to 100 ml of H_2O to make a final concentration of 0.1% (v/v). Incubate at 37°C for at least 12 hours and then autoclave at 15 psi on liquid cycle for 20 minutes to inactivate the remaining DEPC.

Note: DEPC reacts with amines. Do not treat Tris buffers with DEPC.

DEPC (see Appendix for Caution)

70% (v/v) Ethanol

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.

Formamide (Deionized)

Reagent-grade formamide can often be used directly, but it decomposes to formic acid and ammonia during storage. Deionized formamide (sometimes called molecular biology grade) can be purchased or prepared as needed. Do not use batches of formamide that have a yellow color.

To deionize formamide, add Dowex XG8 mixed-bed resin to **formamide** in a glass beaker and stir gently with a magnetic stirring bar for 1 hour. Filter through Whatman No. 1 paper to remove the resin. (Dowex XG8 resin can be purchased with a pH-sensitive blue indicator that becomes yellow upon removal of the acid from the formamide.) Divide into small aliquots and store under nitrogen (to prevent oxidation) at −80°C. Sterilization is not required.

formamide (see Appendix for Caution)

80% (v/v) Glycerol

Carefully measure 80 ml of glycerol in a nonwetting plastic graduated cylinder and transfer it into a 250-ml glass bottle or flask. Add 20 ml of H_2O and stir with a magnetic stirring bar until homogeneous. Sterilize by autoclaving.

PBS

Historically, this commonly used reagent was sodium phosphate-buffered saline (150 mM NaCl plus 10 mM sodium phosphate). PBS has been modified to suit different applications. D-PBS (8 mM sodium phosphate, 2 mM potassium phosphate, 140 mM NaCl, 2.7 mM KCl [pH 7.4] with or without 0.5 mM $MgCl_2$ and 0.9 mM $CaCl_2$) can be purchased as 1x and 10x stock buffers. One preparation of PBS is provided here.

Component and final concentration	Amount to add per 1 liter
137 mM NaCl	8 g
2.7 mM KCl	200 mg
10 mM Na_2HPO_4 (dibasic, anhydrous)	1.44 g
2 mM KH_2PO_4 (monobasic, anhydrous)	240 mg
H_2O	to make 1 liter

Dissolve the components in approximately 0.9 liter of H_2O. Adjust the pH to 7.4 with **HCl** and then adjust the final volume to 1 liter with H_2O.

concentrated HCl (see Appendix for Caution)

Phosphate Buffers

Mixing 1 M NaH_2PO_4 (monobasic) and 1 M Na_2HPO_4 (dibasic) stock solutions in the volumes designated in the table below results in 1 liter of 1 M sodium phosphate buffer of the desired pH. To prepare the 1 M stock solutions, dissolve 138 g of $NaH_2PO_4 \cdot H_2O$ (monobasic; m.w. = 138 g/mole) in sufficient H_2O to make a final volume of 1 liter and dissolve 142 g of Na_2HPO_4 (dibasic, anhydrous; m.w. = 142 g/mole) in sufficient H_2O to make a final volume of 1 liter.

Volume of 1 M NaH_2PO_4 (ml)	Volume of 1 M Na_2HPO_4 (ml)	Final pH
877	123	6.0
850	150	6.1
815	185	6.2
775	225	6.3
735	265	6.4
685	315	6.5
625	375	6.6
565	435	6.7
510	490	6.8
450	550	6.9
390	610	7.0
330	670	7.1
280	720	7.2

TE

Component and final concentration	Amount to add per 100 ml
10 mM Tris-Cl	1 ml of 1 M (pH 7.4–8.0 at 25°C)
1 mM EDTA	200 µl of 0.5 M (pH 8.0)
H_2O	98.8 ml

This standard buffer is used to resuspend and store DNA. It can be prepared by using 1 M Tris-Cl at pHs ranging from 7.4 to 8.0.

Tris-Cl Buffers

Mixing the volumes of concentrated HCl (11.6 N) designated in the table below with 121 g of Tris base (m.w. = 121 g/mole) results in 1 liter of 1 M Tris-Cl buffer of the desired pH at 25°C. To prepare the 1 M buffer, dissolve 121 g of Tris base in approximately 0.9 liter of H_2O. Add the appropriate volume of **concentrated HCl** and adjust the final volume to 1 liter with H_2O.

Volume of concentrated HCl (ml)	pH
8.6	9.0
14	8.8
21	8.6
28.5	8.4
38	8.2
46	8.0
56	7.8
66	7.6
71.3	7.4
76	7.2

Notes: Some pH electrodes cannot accurately measure the pH of Tris solutions. Be sure to check the information provided by the electrode manufacturer.

Tris has a significant temperature coefficient. As the temperature of the solution decreases from 25°C to 5°C, the pH increases an average of 0.03 pH units per degree centigrade. Conversely, as the temperature increases from 25°C to 37°C, the pH decreases an average of 0.025 pH units per degree centigrade. Slight effects on pH based on the total Tris concentration have also been noted. Most molecular biology protocols do not account for these effects.

concentrated HCl (see Appendix for Caution)

ELECTROPHORESIS BUFFERS, DYES, AND GEL-LOADING SOLUTIONS

50x TAE buffer

Component and final concentration	Amount to add per 1 liter
2 M Tris base	242 g
1 M acetate	57.1 ml of **glacial acetic acid** (17.4 M)
100 mM EDTA	200 ml of 0.5 M (pH 8.0)
H_2O	to make 1 liter

This buffer does not have the buffering capacity of TBE buffer. The 1x TAE buffer (pH 8.1) is 40 mM Tris, 20 mM acetate, and 2 mM EDTA.

glacial acetic acid (see Appendix for Caution)

5x TBE buffer

Component and final concentration	Amount to add per 1 liter
445 mM Tris base	54 g
445 mM borate	27.5 g of boric acid
10 mM EDTA	20 ml of 0.5 M (pH 8.0)
H_2O	to make 1 liter

TBE can be prepared as a 5x or 10x stock buffer, but the 10x stock buffer will precipitate during storage. The 1x buffer (pH 8.3) is 89 mM Tris, 89 mM borate, and 2 mM EDTA.

1% Bromophenol Blue

Add 1 g of the H_2O-soluble sodium form of bromophenol blue to 100 ml of H_2O. Stir or mix by vortexing until fully dissolved. In general, this solution is not sterilized.

10 mg/ml Ethidium Bromide

Since this chemical is commonly used in a molecular biology laboratory, a 100-ml stock solution can be prepared. Carefully weigh 1 g of **ethidium bromide**, avoiding dispersal of the powder. Transfer into a wide-mouth bottle and add 100 ml of H_2O and a magnetic stirring bar. Stir until dissolved. Wrap with foil and store at 4°C.

Use of ethidium bromide powder can be completely avoided by purchasing commercially available solutions or by dissolving one 100-mg tablet of ethidium bromide (Sigma E 2515) in 10 ml of H_2O. Sterilization is not required.

ethidium bromide (see Appendix for Caution)

1% Xylene Cyanole FF

Dissolve 1 g of xylene cyanole FF in sufficient H_2O to make a final volume of 100 ml. In general, this solution is not sterilized.

Gel-loading Solutions

Gel-loading solutions added to DNA samples for analysis on agarose or acrylamide gels can contain sucrose, glycerol, or Ficoll as the agent to increase the density of the sample. A dense sample drops evenly to the bottom of a gel well. Individual preference dictates which to use.

Tracking dyes indicate the extent of electrophoresis. Bromophenol blue migrates at the position of an approximately 300-bp linear double-stranded DNA and xylene cyanole FF migrates at the position of a 4-kb linear double-stranded DNA in 0.5x TBE buffer. The preparations below use dye markers at a final concentration of 0.15–0.25%. In general, these gel-loading solutions are not sterilized. Add 2 µl of 6x buffer per 10 µl of total sample or 1 µl of 10x buffer to 9 µl of total sample.

6x Alkaline Gel-loading Solution

Component and final concentration	Amount to add per 10 ml
0.3 N **NaOH**	300 µl of 10 N
6 mM EDTA	120 µl of 0.5 M (pH 8.0)
18% Ficoll (Type 400)	1.8 g
0.15% bromocresol green	15 mg
0.25% xylene cyanole FF	25 mg
H_2O	to make 10 ml

Store at room temperature.

NaOH (see Appendix for Caution)

6x Ficoll Gel-loading Solution

Component and final concentration	Amount to add per 10 ml
0.15% bromophenol blue	1.5 ml of 1%
0.15% xylene cyanole FF	1.5 ml of 1%
5 mM EDTA	100 µl of 0.5 M (pH 8.0)
15% Ficoll (Type 400)	1.5 g
H_2O	to make 10 ml

Store at room temperature.

6x BP/XC/Ficoll Gel-loading Solution

Component and final concentration	Amount to add per 10 ml
0.25% bromophenol blue	2.5 ml of 1%
0.25% xylene cyanole FF	2.5 ml of 1%
15% Ficoll (Type 400)	1.5 g
H_2O	to make 10 ml

Store at room temperature.

Formamide/EDTA Gel-loading Solution

Component and final concentration	Amount to add per 10 ml
98% deionized **formamide**	9.8 ml
10 mM EDTA	200 µl of 0.5 M (pH 8.0)

Divide into 1-ml aliquots and store at –20°C.

Notes: For fluorescent automated DNA sequence analysis, it is not necessary to add tracking dyes to the gel-loading solution.

For radioactive DNA sequence analysis, the presence of 0.025% bromophenol blue and 0.025% xylene cyanole FF is desirable. Add 2.5 mg of each dye per 10 ml of gel-loading solution.

formamide (see Appendix for Caution)

6X Glycerol Gel-loading Solution

Component and final concentration	Amount to add per 10 ml
0.15% bromophenol blue	1.5 ml of 1%
0.15% xylene cyanole FF	1.5 ml of 1%
5 mM EDTA	100 µl of 0.5 M (pH 8.0)
30% glycerol	3 ml
H_2O	3.9 ml

Store at 4°C.

BP/XC/Glycerol Gel-loading Solution

Component and final concentration	Amount to add per 10 ml
0.25% bromophenol blue	2.5 ml of 1%
0.25% xylene cyanole FF	2.5 ml of 1%
30% glycerol	3 ml
H_2O	2 ml

Store at room temperature.

10X SDS/Glycerol Gel-loading Solution

Component and final concentration	Amount to add per 10 ml
200 mM EDTA	4 ml of 0.5 M (pH 8.0)
0.1% **SDS**	100 µl of 10%
50% glycerol	5 ml
0.2% bromophenol blue	20 mg
0.2% xylene cyanole FF	20 mg
H_2O	to make 10 ml

Store this denaturing gel-loading solution at room temperature.

SDS (see Appendix for Caution)

6x Sucrose Gel-loading Solution

Component and final concentration	Amount to add per 10 ml
0.15% bromophenol blue	1.5 ml of 1%
0.15% xylene cyanole FF	1.5 ml of 1%
5 mM EDTA	100 µl of 0.5 M (pH 8.0)
40% sucrose	4 g
H_2O	to make 10 ml

Store at 4°C.

MEDIA

All preparations are for 1 liter of medium. Autoclave prepared liquid medium in the final flask in which cultures will be grown (ideally, a flask that is at least five times the volume of the medium to allow adequate aeration during growth) or divide into aliquots in conveniently sized bottles.

Wear a face mask to avoid inhalation of the fine powders. Use extreme caution in handling autoclaved medium. Wear thermal gloves and do not swirl hot solutions. Overheating can cause boiling medium to bubble out of containers.

Media Preparation

In general, add reagents to 0.9 liter of H_2O. Shake in a flask (at least a 2-liter flask) or stir with a magnetic stirring bar until dissolved. Adjust the pH if required. Adjust the final volume to 1 liter with H_2O.

Plate Preparation

Add reagents to 0.9 liter of H_2O. Shake in a flask (at least a 2-liter flask) or stir with a magnetic stirring bar until dissolved (agar will not completely dissolve until it is autoclaved). Adjust the pH if required. Adjust the final volume to 1 liter with H_2O. Cover the flask loosely with aluminum foil or a suitable cap. Sterilize by autoclaving. Wear thermal gloves and *carefully* swirl the solution to mix thoroughly. Allow the solution to cool in a water bath set at 55°C. Pour approximately 25–30 ml of the cooled solution into each 10-cm plastic petri dish or approximately 100 ml into each 15-cm plastic petri dish. Use a Bunsen burner to flame the surface of the medium in the plate to remove bubbles. Allow the plates to solidify at room temperature. Store plates upside down at 4°C.

A convenient alternative is to prepare medium in glass bottles and allow it to solidify. Store the solidified medium at room temperature. When needed, melt the solid medium in a microwave oven and pour plates.

Top Agar Preparation

Add reagents to 0.9 liter of H_2O. Shake in a flask (at least a 2-liter flask) or stir with a magnetic stirring bar until dissolved. Adjust the pH if required. Adjust the final volume to 1 liter with H_2O. If desired, divide the medium into 100-ml aliquots in autoclavable bottles before adding the agar and autoclaving. Add the appropriate amount of agar to the medium (for bacterial top agar, add 0.7 g per 100 ml) and then sterilize by autoclaving. Allow the top agar to solidify at room temperature.

Carefully melt the top agar in a microwave oven or in a boiling-water bath. Be sure to loosen caps on bottles before heating them. Do not leave top agar unattended in a microwave oven; the medium can easily bubble over with overheating or swirling of the hot solution. One approach to dealing with boiling over during melting is to break up solidified top agar with a sterile pipette before heating it in a microwave oven. Allow the top agar to cool to 45–48°C before use.

Bacterial/Bacteriophage Media

LB MEDIUM

Combine the following in 0.9 liter of H_2O:

Bacto tryptone	10 g
Bacto yeast extract	5 g
NaCl	10 g

Adjust the pH to 7.0 with 1 N **NaOH** (~1 ml) if desired. Adjust the final volume to 1 liter with H_2O.

Note: For agar plates, include 12 g of Bacto agar per liter. For top agar, include 7 g of Bacto agar per liter.

NaOH (see Appendix for Caution)

SOB MEDIUM

Combine the following in 0.9 liter of H_2O:

Bacto tryptone	20 g
Bacto yeast extract	5 g
NaCl	0.5 g
1 M KCl	2.5 ml

Adjust the final volume to 1 liter with H_2O. Divide the medium into 100-ml aliquots and then sterilize by autoclaving. Allow the medium to cool to room temperature, and then add 1 ml of sterile 1 M $MgCl_2$ to each 100-ml aliquot.

SOC MEDIUM

Prepare SOC medium as described for SOB medium but add 2 ml of sterile 1 M glucose to each 100-ml aliquot (18 g of glucose dissolved in sufficient H_2O to make a final volume of 100 ml; sterilize the solution by passing it through a 0.22-μm filter) in addition to the 1 ml of 1 M $MgCl_2$ after the medium has cooled to room temperature.

TB MEDIUM

Combine the following in sufficient H_2O to make 0.9 liter:

Bacto tryptone	12 g
Bacto yeast extract	24 g
glycerol	4 ml

Dissolve the components and then sterilize by autoclaving. Allow to cool to at least 60°C. Add 100 ml of a sterile solution of 170 mM KH_2PO_4/0.72 M K_2HPO_4

(2.31 g of KH_2PO_4 [monobasic, anhydrous] and 12.54 g of K_2HPO_4 [dibasic, anhydrous] in sufficient H_2O to make a final volume of 100 ml; sterilize the solution by passing it through a 0.22-µm filter or by autoclaving).

2x YT MEDIUM

Combine the following in 0.9 liter of H_2O:

Bacto tryptone	16 g
Bacto yeast extract	10 g
NaCl	5 g

Adjust the pH to 7.0 with 1 N **NaOH** (~1 ml) if desired. Adjust the final volume to 1 liter with H_2O.

Note: For agar plates, include 12 g of Bacto agar per liter. For top agar, include 7 g of Bacto agar per liter.

NaOH (see Appendix for Caution)

Yeast Media

AHC MEDIUM

Combine the following in 0.8 liter of H_2O:

YNB without amino acids (Difco 0919-15)	6.7 g
casein acid hydrolysate, low salt (USB 12852)	10 g
adenine hemisulfate monohydrate (Sigma A 9126)	20 mg

Adjust the pH to 5.8 with **HCl**. Adjust the final volume to 0.95 liter with H_2O. Sterilize by autoclaving. Allow to cool. Add 50 ml of sterile 40% glucose (40 g of glucose dissolved in sufficient H_2O to make a final volume of 100 ml; sterilize the solution by passing it through a 0.22-µm filter or by autoclaving) per liter and mix well.

Notes: To prepare plates, add 20 g of Bacto agar before autoclaving. Agar from Difco is highly recommended, otherwise a precipitate may form upon autoclaving.

Instead of the YNB without amino acids used above, 1.7 g of YNB without ammonium sulfate or amino acids (Difco 0335-15-9) plus 5 g of ammonium sulfate can be substituted in each liter.

For high-adenine AHC medium, prepare liquid or solid medium as described above but add 50–100 mg (instead of 20 mg) of adenine hemisulfate to each liter. The higher adenine concentration in high-adenine AHC medium allows Ade^- strains (which includes many YAC-containing strains) to grow faster and to reach a higher cell density (but note that certain Ade^- strains, e.g., *ade2*, will not develop the characteristic red color).

For AHC + Trp medium, add 4 ml of sterile 1% tryptophan per liter after autoclaving.

concentrated HCl (see Appendix for Caution)

SC MEDIUM AND "DROP OUT" MEDIA

Combine the following in sufficient H_2O to make 0.9 liter:

YNB without amino acids (Difco 0919-15)	6.7 g
powdered supplement mixture	2 g

Sterilize by autoclaving. Allow to cool. Add 100 ml of 20% glucose (20 g of glucose dissolved in sufficient H_2O to make a final volume of 100 ml; sterilize the solution by passing it through a 0.22-μm filter or by autoclaving) and mix well.

Notes: To prepare plates, add 20 g of Bacto agar before autoclaving.

Instead of the YNB without amino acids used above, 1.7 g of YNB without ammonium sulfate or amino acids (Difco 0335-15-9) plus 5 g of ammonium sulfate can be substituted in each liter.

Solid or liquid SC medium contains all supplements listed in the table below. These supplements are added in the form of a well-mixed powder (2 g of powder per 1 liter of medium). SC medium can be made selective for the growth of the desired prototrophs by dropping out ingredients. For example, "drop out" medium referred to as SC – Ura,Trp contains all powdered supplements except uracil and tryptophan.

Powdered supplement mixture for SC medium or "drop out" media

Supplement	Amount to add
adenine (hemisulfate salt)	0.5 g
L-alanine	2 g
L-arginine HCl	2 g
L-asparagine (monohydrate)	2 g
L-aspartic acid	2 g
L-cysteine HCl	2 g
glutamine	2 g
L-glutamic acid (monosodium salt)	2 g
glycine (sodium salt)	2 g
L-histidine HCl	2 g
myo-inositol	2 g
L-isoleucine	2 g
L-leucine	4 g
L-lysine HCl	2 g
L-methionine	2 g
p-aminobenzoic acid	0.2 g
L-phenylalanine	2 g
L-proline	2 g
L-serine	2 g
L-threonine	2 g
L-tryptophan	2 g
L-tyrosine	2 g
uracil	2 g
L-valine	2 g

Before adding supplements to the medium, thoroughly mix the powdered supplements in a plastic bottle by adding several clean glass marbles and shaking vigorously.

YPD MEDIUM

Combine the following in 0.9 liter of H_2O:

Bacto peptone	20 g
Bacto yeast extract	10 g
glucose	20 g

Adjust the final volume to 1 liter with H_2O. Sterilize by autoclaving.

Notes: Adding supplemental tryptophan (1.6 g of tryptophan per liter) before autoclaving is recommended for Trp^- auxotrophs since YPD medium is limiting for tryptophan.

Adding supplemental adenine (50 mg of adenine hemisulfate monohydrate per liter) before autoclaving is recommended for Ade^- auxotrophs.

To prepare plates, add 20 g of Bacto agar before autoclaving.

ANTIMICROBIAL AGENTS

Whenever possible, purchase the H_2O-soluble salt form (sodium, hydrochloride, or sulfate) of antimicrobial agents. All stock solutions are prepared with either sterile H_2O or absolute ethanol. No additional sterilization is needed because of the nature of the chemicals. The small volume of ethanol added to media or plates is of no consequence.

Ampicillin (100 mg/ml)

Dissolve 1 g of sodium ampicillin in sufficient H_2O to make a final volume of 10 ml. Divide into aliquots and store at –20°C.

Ampicillin, a penicillin derivative, is bactericidal only to growing cells. It inhibits cell wall biosynthesis by preventing cross-linking of the peptidoglycan. β-Lactamase, which is encoded by the *bla* gene, confers resistance by cleaving ampicillin's β-lactam ring. Ampicillin is typically used in growth medium at a final concentration of 25–50 μg/ml.

Carbenicillin (50 mg/ml)

Dissolve 0.5 g of disodium carbenicillin in sufficient H_2O to make a final volume of 10 ml. Divide into aliquots and store at –20°C.

Like ampicillin above, carbenicillin is a penicillin derivative and is typically used in growth medium at a final concentration of 25–50 μg/ml.

Chloramphenicol (25 mg/ml)

Dissolve 250 mg of chloramphenicol in sufficient absolute ethanol to make a final volume of 10 ml. Divide into aliquots and store at –20°C.

Chloramphenicol is bacteriostatic because it inhibits protein synthesis. Chloramphenicol transacetylase, which is encoded by the *cam* gene, confers resistance by acetylating chloramphenicol and thus preventing its inhibitory activity. Chloramphenicol is typically used in growth medium at a final concentration of 12.5–25 μg/ml. For complete inhibition of host protein synthesis, use a final concentration of 170 μg/ml.

Kanamycin (10 mg/ml)

Dissolve 100 mg of kanamycin monosulfate in sufficient H_2O to make a final volume of 10 ml. Divide into aliquots and store at –20°C.

Kanamycin is bactericidal because it inhibits 70S ribosomal subunit translocation during protein synthesis. Aminoglycoside-modifying enzymes confer resistance by modifying kanamycin and thus preventing its inhibitory activity. Kanamycin is typically used in growth medium at a final concentration of 10–50 μg/ml.

Methicillin (100 mg/ml)

Dissolve 1 g of sodium methicillin in sufficient H_2O to make a final volume of 10 ml. Divide into aliquots and store at –20°C.

Methicillin is a penicillin derivative that is used to prevent the formation of satellite colonies during selection with ampicillin. Methicillin can be used in growth medium at a final concentration of 37.5 μg/ml in combination with ampicillin at a final concentration of 100 μg/ml.

Nalidixic Acid (5 mg/ml)

Dissolve 50 mg of the sodium salt of nalidixic acid in sufficient H_2O to make a final volume of 10 ml. Divide into aliquots and store at –20°C.

Nalidixic acid is bacteriostatic because it inhibits DNA synthesis through its action on DNA gyrase. Mutations in the gyrase gene confer resistance. Nalidixic acid is typically used in growth medium at a final concentration of 15 μg/ml.

Streptomycin (50 mg/ml)

Dissolve 0.5 g of streptomycin sulfate in sufficient absolute ethanol to make a final volume of 10 ml. Divide into aliquots and store at –20°C.

Streptomycin is bactericidal because it acts on the S12 protein of the 30S ribosomal subunit, thereby inhibiting protein synthesis. Mutations in the gene encoding the S12 protein (*rpsL*) confer resistance by preventing binding of streptomycin. Inactivation by aminoglycoside phosphotransferase can also occur. Streptomycin is typically used in growth medium at a final concentration of 10–50 μg/ml.

Tetracycline (10 mg/ml)

Dissolve 100 mg of tetracycline hydrochloride in sufficient H_2O to make a final volume of 10 ml. Alternatively, dissolve the free base form of tetracycline in absolute ethanol. Divide into aliquots and store in foil-wrapped tubes at –20°C to protect the solution from light.

Tetracycline is bacteriostatic because it inhibits elongation during protein synthesis. It also prevents binding of aminoacyl tRNA to the ribosome. Loss of cell wall permeability confers resistance to tetracycline. Tetracycline is typically used in growth medium at a final concentration of 10–50 μg/ml.