Recipes for Common Laboratory Solutions

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Solution	Preparation
agarose gel sample buffer (6X)	Dissolve 4g sucrose and 2.5mg bromophenol blue in 6ml of TE buffer [10mM Tris-HCl (pH 8.0), 1mM EDTA]. Once dissolved, bring up to a final volume of 10ml with TE buffer. Store at room temperature.
7.5M ammonium acetate	Dissolve 57.81g of ammonium acetate in water to a final volume of 100ml. Sterilize by filtration (0.2µm filter). The final pH will be 5.5.
BigDye® dilution buffer	250mM Tris-HCl (pH 9.0), 10mM $MgCl_2$.
D-biotin, 100mM	Dissolve desired amount of biotin in 100mM Na_2HPO_4. Adjust pH to 7.2 with 100mM NaH_2PO_4. Bring to desired volume with 100mM sodium phosphate buffer (pH 7.2). Filter-sterilize (0.22µm filter), and dispense under aseptic conditions.
Denhardt's solution (50X)	Dissolve 5g Ficoll®, 5g polyvinylpyrrolidone and 5g BSA in 300ml of water. Once dissolved, bring up to 500ml with water. Filter, and dispense into 25ml aliquots. Store at –20°C.
DEPC treatment	Add 0.2ml of DEPC (diethylpyrocarbonate) to 100ml solution to be treated. Shake vigorously, and allow the solution to sit overnight in a fume hood. 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. Do not treat solutions containing primary amines, such as Tris, with DEPC.
1M dithiothreitol (DTT)	Dissolve 3.09g DTT in 20ml of 0.01M sodium acetate (pH 5.2). Sterilize by filtration. Dispense into 1ml aliquots, and store at –20°C.
0.5M EDTA (pH 8.0)	Add 186.1g disodium ethylenediamine tetraacetate•$2H_2O$ to 800ml of water. Stir vigorously on a magnetic stirrer. Adjust the pH to 8.0 with NaOH. EDTA will slowly go into solution as the pH approaches 8.0. Dispense into aliquots, and sterilize by autoclaving.
ethidium bromide, 10mg/ml	Add 1g ethidium bromide to 100ml of water. Stir on a magnetic stirrer for several hours to ensure that the dye has dissolved. Wrap the container in aluminum foil or transfer to a dark bottle, and store at 4°C. **Caution:** Ethidium bromide is a mutagen and is toxic. Wear gloves when working with ethidium bromide solutions and a mask when weighing the powder.
IPTG	Dissolve 1.2g IPTG (isopropyl-β-D-thiogalactopyranoside) in deionized water to a final volume of 50ml. Filter sterilize (0.2µm), and store in 5ml aliquots at –20°C. The final concentration of IPTG is 0.1M. The stock solution of IPTG is stable at this temperature for 2–4 months.
LB	Add 10g Bacto®-tryptone, 5g Bacto®-yeast extract and 5g NaCl to deionized water to a final volume of 1 liter. Adjust the pH to 7.5 with 10N NaOH, and autoclave to sterilize. Store at room temperature in 500ml aliquots.
5X MOPS gel running buffer	To prepare 2 liters of buffer, add 83.72g MOPS (free acid) and 8.23g sodium acetate to 1.6 liters of DEPC-treated water, and stir until completely dissolved. Add 20ml of DEPC-treated 0.5M EDTA, and adjust the pH to 7.0 with 10N NaOH. Bring the final volume to 2 liters with DEPC-treated water. Dispense into 200ml aliquots, and autoclave. The solution turns yellow, but this will not affect the quality of the buffer. Store aliquots protected from light at room temperature or 4°C.
M9 plates with 1mM thiamine-HCl	Add 6g Na_2HPO_4, 3g KH_2PO_4, 0.5g NaCl, 1g NH_4Cl, and 15g agar to deionized water to a final volume of 1 liter. Adjust the pH to 7.4 with 10N NaOH. Autoclave and cool to 50°C, then add: 2.0ml 1M $MgSO_4$, 0.1ml 1M $CaCl_2$, 10ml 20% glucose, 1.0ml 1M thiamine-HCl. Filter the complete medium through a 0.2µm filter unit to sterilize the medium, then pour into plates. Store inverted covered plates at 4°C, where they are stable for up to 1–2 months.
Mueller Hinton II Broth	Add 300g beef infusion, 17.5g Bacto® casamino acids, 1.5g Bacto® soluble starch to deionized water to a final volume of 1 liter. Adjust the pH to 7.3, and autoclave to sterilize.
Mueller Hinton II Broth (cation-adjusted)	Prepare a magnesium stock solution by dissolving 8.36g of $MgCl_2$•$6H_2O$ in 100ml of deionized water; the final concentration is 10mg/ml Mg^{2+}. Prepare a calcium stock solution by dissolving 3.68g of $CaCl_2$•$2H_2O$ in 100ml of deionized water; the final concentration is 10mg/ml Ca^{2+}. Filter-sterilize both stock solutions. To Mueller Hinton II broth, add the magnesium stock solution to a final concentration of 10–12.5mg/L Mg^{2+}. Add the calcium stock solution to a final concentration of 20–25mg/L Ca^{2+}.
phenol (acid) (for RNA only)	Heat to 55°C to dissolve 500g phenol in 500ml of 50mM sodium acetate (pH 4.0). Once dissolved, let phases separate and remove upper aqueous phase. Add 500ml of 50mM sodium acetate (pH 4.0), and stir to emulsify. Let phases separate. Repeat procedure until the pH of the upper aqueous phase is less than 4.1.
phosphate-buffered saline (PBS)	Dissolve 8g NaCl, 0.2g KCl, 1.44g Na_2HPO_4 and 0.24g KH_2PO_4 in 800ml of distilled water. Adjust to pH 7.4 with HCl. Add water to 1 liter. Dispense into aliquots. Sterilize by autoclaving.
potassium acetate for alkaline lysis	Add 11.5ml of glacial acetic acid and 28.5ml of water to 60ml of 5M potassium acetate. The resulting solution is 3M with respect to potassium and 5M with respect to acetate.
RNA loading buffer	Prepare in DEPC-treated water, 50% glycerol, 1mM EDTA, 0.4% bromophenol blue and 1mg/ml ethidium bromide. Use a high-grade glycerol to avoid ribonuclease contamination. Dispense into 500µl aliquots, and store at –20°C. Use 2µl of loading buffer per 10–20µl of RNA sample (RNA plus sample buffer).
RNA sample buffer	Combine 10.0ml of deionized formamide, 3.5ml of 37% formaldehyde and 2.0ml of 5X MOPS. Mix thoroughly, dispense into 500µl aliquots and store at –20°C in tightly sealed screw-cap tubes. The buffer can be stored for up to 6 months at this temperature. Use 2 parts sample buffer for each part of RNA. **Caution:** Formamide is a teratogen, and formaldehyde is a toxic carcinogen. Work in a fume hood, and follow standard laboratory safety procedures.
SDS gel sample buffer (2X)	Combine 20ml of glycerol, 5ml of β-mercaptoethanol, 20ml of 10% SDS, 20mg bromophenol blue and 25ml of 4X stacking gel buffer (6.06g Tris, 4ml of 10% SDS, bring to a final pH of 6.8 and bring to 100ml with water). Dissolve, and bring to 100ml with water. Adjust pH to 6.8 with 12N HCl. Store at room temperature.

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Recipes for Common Laboratory Solutions (continued).

Solution	Preparation
10% sodium dodecyl sulfate (SDS)	Dissolve 100g electrophoresis-grade SDS in 900ml of water. Heat to 68°C to assist dissolution. Adjust the pH to 7.2 with HCl. Adjust volume to 1 liter. Dispense into aliquots. SDS is an irritant; wear a mask while weighing the powder.
20X SSC	Dissolve 175.3g NaCl and 88.2g sodium citrate in 800ml of water. Adjust pH to 7.0 with 10N NaOH. Adjust volume to 1 liter. Dispense into aliquots. Sterilize by autoclaving.
20X SSPE	Dissolve 175.3g NaCl, 27.6g $NaH_2PO_4 \bullet H_2O$ and 7.4g EDTA in 800ml of water. Adjust pH to 7.4 with NaOH (approximately 6.5ml of a 10N NaOH solution). Adjust volume to 1 liter. Dispense into aliquots. Sterilize by autoclaving.
SOC	Add 2.0g Bacto®-tryptone, 0.5g Bacto®-yeast extract, 1ml of 1M NaCl and 0.25ml of 1M KCl to 97ml of deionized water, and stir until dissolved. Autoclave, and cool to room temperature. Add 1ml of 2M magnesium stock (1M $MgCl_2$, 1M $MgSO_4$) and 1ml of 2M glucose stock, each to a final concentration of 20mM. Filter the complete medium through a 0.2µm filter unit to sterilize. The pH should be 7.0. Store at room temperature in 25–50ml aliquots.
50X TAE	Dissolve 242g Tris base and 37.2g $Na_2EDTA \bullet (2H_2O)$ in 900ml of deionized water. Add 57.1ml of glacial acetic acid, and adjust the final volume with water to 1 liter. Store at room temperature or 4°C.
10X TBE	Dissolve 108g Tris base and 55g boric acid in 900ml of deionized water. Add 40ml of 0.5M EDTA (pH 8.0), and increase the final volume to 1 liter. Store at room temperature or 4°C.
trichloroacetic acid (TCA) 100% solution	Add 227ml of water to a bottle containing 500g TCA. The solution will be 100% (w/v) TCA.
1X TE buffer (pH 8.0)	10mM Tris-HCl (pH 8.0), 1mM EDTA.
TE^{-4} buffer (pH 8.0)	Dissolve 2.21g Tris base and 0.037g EDTA (Na_2 $EDTA \bullet 2H_2O$) in 900ml of deionized water. Adjust to pH 8.0 with HCl. Bring the volume to 1 liter with deionized water.
TE-saturated phenol:chloroform:isoamyl alcohol	Dissolve 500g phenol in 500ml of chloroform. Once dissolved, add 25ml of isoamyl alcohol. Add 1 volume of 10mM Tris-HCl (pH 8.0), 1mM EDTA (TE buffer). Stir to emulsify. Let phases separate. Remove top phase, and repeat TE buffer addition. Let phases separate, removing all but 1cm of aqueous phase. Store in a dark bottle at 4°C.
urea polyacrylamide gel sample buffer	Dissolve 10g sucrose, 20mg bromophenol blue and 20mg xylene cyanol in 90ml of deionized formamide. Bring up to a final volume of 100ml with water.
X-Gal	Dissolve 100mg X-Gal in N, N′-dimethylformamide to a final volume of 2ml. Dispense into 500µl aliquots, and store protected from light at –20°C. The final concentration of X-Gal is 50mg/ml. The stock solution of X-Gal should be stable for 2–4 months at this temperature.

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