STOCK SOLUTIONS

5 M NaCl (F.W. 58.44) 500 ml:
146.1 g NaCl
~350 ml H_2O
Dissolve, then bring up to volume with H_2O
Sterilize by autoclaving (15 minutes)

1 M Tris, pH 8 (F.W. 121.1) 500 ml:
60.55 g Trizma base
~400 ml H_2O
Approximately 21.1 ml concentrated HCl (Use pH meter)
Bring up to volume with H_2O
Sterilize by autoclaving (15 minutes)

1 M Tris, pH 7.6 (F.W. 121.1) 500 ml:
60.55 g Trizma base
~400 ml H_2O
Approximately 28.5 ml concentrated HCl (Use pH meter)
Bring up to volume with H_2O
Sterilize by autoclaving (15 minutes)

1 M $MgCl_2$ (F.W. 203.30) 100 ml:
20.33 g $MgCl_2$
~70 ml H_2O
Dissolve, then bring up to volume with H_2O
Sterilize by autoclaving (15 minutes)

1 M $CaCl_2$ (F.W. 147.02) 100 ml:
14.70 g $CaCl_2$
~90 ml H_2O
Dissolve, then bring up to volume with H_2O
Sterilization not required

1 M $MgSO_4$ (F.W. 246.47) 100 ml:
24.65 g $MgSO_4$
~75 ml H_2O
Dissolve, then bring up to volume with H_2O
Sterilize by autoclaving (15 minutes)

0.1 M $MgCl_2$ (F.W. 203.30) 100 ml:
2.03 g $MgCl_2$
~95 ml H_2O
Dissolve, then bring up to volume with H_2O
Sterilize by autoclaving (15 minutes)

3 M NaOH (F.W. 40.00) 100 ml:
12.0 g NaOH
~80 ml H_2O
Dissolve, then bring up to volume with H_2O
Sterilization not required

2 M Sorbitol (F.W. 182.2) 500 ml:
182.2 g Sorbitol
~300 ml H_2O
Dissolve, then bring up to volume with H_2O
Sterilization by filtration

5 M Potassium acetate (F.W. 98.14) 100 ml:
49.07 g Potassium acetate
~70 ml H_2O
Dissolve, then bring up to volume with H_2O
Sterilize by autoclaving (15 minutes)

3 M Potassium / 5 M Acetate
To prepare 1 liter of this solution, dissolve 294.42 g potassium acetate in 100 ml water, and add glacial acetic acid until a pH of 4.6 is reached. This will require about 40-50% of the final volume to be acetic acid. Bring to 1 liter final volume. It is very important that the pH of this solution be correct.

0.5 M Na_2HPO_4 (F.W. 141.96) 500 ml
35.49 g $NaPO_4$
~450 ml H_2O
Dissolve, then bring up to volume with H2O
Sterilize by autoclaving (15 minutes)

0.5 M NaH_2PO_4 (F.W. 137.99) 500 ml
34.50 g NaH_2PO_4
~450 ml H_2O
Dissolve, then bring up to volume with H_2O
Sterilize by autoclaving (15 minutes)

3 M Sodium acetate (F.W. 136.10) 500 ml
204.15 g Sodium acetate
~200 ml H_2O
90 ml Glacial Acetic Acid
Dissolve, then bring up to volume with H_2O
Sterilize by autoclaving (15 minutes)

0.5 M EDTA, pH 8.0 (F.W. 336.2) 500 ml
84.05 g EDTA
~250 ml H_2O

EDTA will not dissolve yet. Add 5 M NaOH slowly with stirring until the EDTA dissolves, and then reaches pH 8.0 (takes approx. 71 ml)
Bring up to volume with H_2O
Sterilize by autoclaving (15 minutes)

0.5 M Sodium Phosphate Buffer (pH 6.5)
500 ml 0.5 M NaH_2PO_4
332.5 ml 0.5 M Na_2HPO_4
Sterilize by autoclaving (15 minutes)

Preparation of 1.0 M Sodium Phosphate Buffer at 25°C
1 M $Na_2HPO_4 \cdot 7H_2O$, 268.07 g per 1 liter
1 M $NaH_2PO4 \cdot H_2O$, 34.45 g per 250 ml

pH	Volume of	
	1M Na_2HPO_4	1M NaH_2PO_4
7.4	774	226
7.5	810	190
7.6	845	155

Other solutions:

STE 500 ml

0.1 M NaCl	10 ml 5 M NaCl
10 mM Tris.Cl (pH 8.0)	5 ml 1 M Tris pH 8.0
1 mM EDTA (pH 8.0)	1 ml 0.5 M EDTA
	Bring to 500 ml with H2O

20´SSC 1 liter
Dissolve 175.3 g of NaCl, and
88.2 g of sodium citrate
in 800 ml of H2O.
Adjust the pH to 7.0 with a few drops 5 or 10 N HCl.
Adjust volume to 1 liter.
Dispense into aliquots.
Sterilize by autoclaving.

20% SDS 1 liter
200 g SDS in 800 ml H_2O
Place in 1 liter bottle on shaker until dissolved (may take overnight)
Adjust pH to 7.2 with 0.5 N HCl

Bring to 1 liter
Maniatis says there is no need to sterilize, but we always autoclave.

Commonly Used Electrophoresis Buffers

Buffer	Working solution	Concentrated stock solution (per liter)
Tris-Acetate (TAE)	1X 0.04 M Tris-acetate 0.001 M EDTA	50X 242 g Tris base 57.1 ml glacial acetic acid 100 ml 0.5 M EDTA (pH 8.0)
Tris-Phosphate (TPE)	1X 0.09 M Tris-phosphate 0.002 M EDTA	10X 108 g Tris base 15.5 ml 85% phosphoric acid (1.679 g/ml) 40 ml 0.5 M EDTA (pH 8.0)
Tris-Borate (TBE) (***Half-Strength*** Formula)	1X 0.045 M Tris-borate 0.001 M EDTA	10X 54 g Tris base 27.5 g boric acid 20 ml 0.5 M EDTA (pH 8.0)

- A precipitate forms when concentrated solutions of TBE are stored for long periods of time. To avoid problems, store the 5´ solution in glass bottles at room temperature and discard any batches that develop a precipitate.

- TBE was originally used at a working strength of 1´ (i.e., a 1:5 dilution of the concentrated stock) for agarose gel electrophoresis. However, a working solution of 0.5´ provides more than enough buffering power, and almost all agarose gel electrophoresis is now carried out with a 1:10 dilution of the concentrated stock.

- TBE is used at a working strength of 1´ for polyacrylamide gel electrophoresis, twice the strength usually used for agarose gel electrophoresis. The buffer reservoirs of the vertical tanks used for polyacrylamide gel electrophores are fairly small, and the amount of electric current passed through them is often considerable. 1´ TBE is required to provide adequate buffering power.

Maniatis 2nd edition, page B.23

ATP

1. Dissolve ATP in H_2O:
   0.1 M 120 mg in 1.6 ml H_2O
   10 mM 60 mg in 8 ml H_2O

2. Adjust the pH to 7.0 with 0.1 M NaOH
3. Adjust the volume to 2 ml (for 0.1 M) or 10 ml (for 10 mM) with H_2O.
4. Dispense into small aliquots (e.g. 20 ml).
5. Store at -70°C.

Dithiothreitol

(DTT, Cleland's Reagent)

HS-CH2-CH-CH-CH2-SH

$\overline{O}H$ $\overline{O}H$

Dithiothreitol reduces sulfides to their corresponding thiols. Dithiothreitol is soluble in water and alcohols and has a lower volatility than that of other reducing agents such as b-mercaptoethanol. At low concentrations DTT can be used in a reaction buffer to maintain enzymatic activities. In higher concentrations DTT dissociates disulfide linkages in polypeptides, which facilitates the denaturation by detergents or chaotropic agents.
Danger: Dithiothreitol is a highly toxic substance. Harmful if inhaled or swallowed. Avoid contact with eyes, skin, and clothing. Use only in a well-ventilated area. Wash thoroughly after handling.

Directions

1. Dissolve DTT in 20 ml distilled H_2O:
   1 Molar 3.09 g
   0.1 Molar 0.309 g

2. Sterilize by filtration (do not autoclave).

3. Dispense into aliquots in microfuge tubes.

4. Store at -20°C.

From BRL ultraPURETM Laboratory Handbook

RNase, DNase Free

1. Dissolve pancreatic RNase (RNase A) at a concentration of 10 mg/ml in 10 mM Tris-Cl (pH 7.5), 15 mM
   For: 1 ml
   1 M Tris 10 ml
   3 M NaCl 5 ml
   RNase 10 mg
   H_2O 985 ml

2. Heat to 100°C for 15 minutes. Allow to cool slowly to room temperature.

3. Dispense into aliquots and store at -20°C.

From Maniatis 2nd Ed. p. B.17