Recipes for stock solutions and general use buffers

How to determine volumes to use to obtain a certain concentration:

- $C_1V_1 = C_2V_2$ where C = concentration and V = volume
- Make sure to match units!
- Example: You have a stock solution at 100 mg/mL. You want 75 mL at 150 μg/mL.
  (100 mg/mL)(x) = (0.150 mg/mL)(75 mL)
  x = 0.1125 mL = 112.5 μL

Things to look up / think about:

Based only on the information in the recipes below, what are the molecular weights (MW) of Tris base, HEPES, and NaCl? What is the molar composition of 20x TBS? What is the atomic weight of Cl?

Understand the units of concentration that are commonly used in the lab: molarity (M, mM, μM, etc.), normality (*N*, whose use is discouraged by IUPAC), weight/volume (g/L, mg/mL, μg/mL, μg/μL, etc.), percent weight or volume (%), and dilution factor (1000x, 10x, 1x, etc.).

Understand the Henderson-Hasselbalch equation.

General use buffers

0.5 M EDTA, pH 8:

For 500 mL

- Resuspend 93.05 g Na_2•EDTA•$2H_2O$ (disodium dyhydrate) in about 400 mL of ddH_2O
- Add about 9 g solid NaOH
- Once all the NaOH dissolves, slowly adjust the pH with 10 *N* NaOH
- Bring up the volume to 500 mL with ddH_2O

Note: EDTA will not completely dissolve until the pH reaches 8

1 M HEPES stocks:

For 1 L

- Dissolve 238.3 g HEPES (free acid) in 800 mL of ddH_2O
- Adjust the pH to the desired value with 10 *N* NaOH
- Bring up the volume to 1 L with ddH_2O

0.5 M MES stocks:

For 1 L

- Dissolve 97.6 g MES (free acid) in 800 mL of ddH_2O
- Adjust the pH to the desired value with 10 *N* NaOH
- Bring up the volume to 1 L with ddH_2O

1 M MOPS stocks:
For 1 L
- Dissolve 209.3 g MOPS (free acid) in 800 mL of ddH_2O
- Adjust the pH to the desired value with 10 *N* NaOH
- Bring up the volume to 1 L with ddH_2O

5 M NaCl:
For 1 L
- Dissolve 292.2 g NaCl in a final volume of 1 L ddH_2O

10 M NaOH (= 10 *N* NaOH):
For 500 mL
- Dissolve 200 g NaOH in a final volume of 500 mL ddH_2O

1 M Tris stocks:
For 1 L
- Dissolve 121.1 g Tris base in 800 mL of ddH_2O
- Adjust the pH to the desired value with concentrated HCl
- Bring up the volume to 1 L with ddH_2O

Buffers for agarose gel electrophoresis

50x TAE:
For 1 L
- Dissolve 242.2 g Tris base in around 600 mL of ddH_2O
- Slowly add 57.1 mL glacial acetic acid
- Add 100 mL 0.5 M EDTA, pH 8
- Bring up the volume to 1 L with ddH_2O

6x DNA loading buffer:
For 100 mL
- Weigh 60 g glycerol into a 100-mL graduated cylinder
- Add 12 mL 0.5 M EDTA, pH 8
- Add 10 mg bromophenol blue
- Bring up the volume to 100 mL with ddH_2O

10x DNA loading buffer:
For 100 mL
- Measure 20 mL 50x TAE into a 100-mL graduated cylinder
- Add 40 g sucrose
- Add 10 mg bromophenol blue
- Bring up the volume to 100 mL with ddH_2O

Buffers for SDS-PAGE

1.5 M Tris, pH 8.8 (stock buffer for separating gels)
For 1 L
- Dissolve 181.65 g Tris base in around 800 mL of ddH_2O

- Adjust the pH to 8.8 with concentrated HCl
- Bring up the volume to 1 L with ddH_2O

Note: Make sure to let the solution cool down to room temperature before making the final pH adjustment.

1.5 M Tris, pH 6.8 (stock buffer for stacking gels)

For 1 L

- Dissolve 181.65 g Tris base in around 700 mL of ddH_2O
- Adjust the pH to 6.8 with concentrated HCl
- Bring up the volume to 1 L with ddH_2O

Note: Make sure to let the solution cool down to room temperature before making the final pH adjustment.

10x Tris-glycine running buffer:

For 4 L

- 121.1 g Tris base
- 576 g glycine
- 200 mL 20% SDS
- Bring up the volume to 4 L with ddH_2O

2x sample loading buffer (non-reducing):

For 100 mL

- 5 mL 1 M Tris, pH 7
- 25 mL 20% SDS
- 20 mL glycerol
- 2 mg bromophenol blue
- Bring up the volume to 100 mL with ddH_2O

2x sample loading buffer (reducing):

For 1 mL

- 950 μL 2x non-reducing sample loading buffer
- 50 μL β-mercaptoethanol

Stain/destain solution:

For 4 L:

- 200 mL absolute ethanol
- 300 mL glacial acetic acid
- Bring up the volume to 4 L with ddH_2O

Fixing solution:

For 1 L:

- 600 mL absolute ethanol
- 75 mL glacial acetic acid
- Bring up the volume to 1 L with ddH_2O

Coomassie Blue stock solution:

For 100 mL:

- Dissolve 250 mg Coomassie Brilliant Blue G-250 (**not R-250**!) in 100 mL of fixing solution

Buffers for western blotting

10x Transfer buffer:
For 4 L
- 121.1 g Tris base
- 576 g glycine
- Bring up the volume to 4 L with ddH_2O

1x Transfer buffer:
For 1 L
- 700 mL cold ddH_2O
- 100 mL 10x Transfer buffer
- 200 mL methanol

20x TBS:
For 4 L
- 193.6 g Tris base
- 640 g NaCl
- Bring up the volume to 3.2 L with ddH_2O
- Adjust the pH to 7.6 with concentrated HCl
- Bring up the volume to 4 L with ddH_2O

20x TBST:
For 100 mL
- Add 2 mL Tween-20 to 100 mL of 20x TBS

E. coli growth media

LB:
For 1 L
- 10 g tryptone
- 5 g yeast extract
- 10 g NaCl
- Optional: Bring up the volume to around 900 mL with ddH_2O, then adjust the pH to 7.4
- Bring up the volume to 1 L with ddH_2O
- Sterilize by autoclaving for 30 min

To make LB-agar, add 15 g of agar prior to autoclaving

Low-salt LB:
For 1 L
- 10 g tryptone
- 5 g yeast extract
- 5 g NaCl
- Optional: Bring up the volume to around 900 mL with ddH_2O, then adjust the pH to 7.4
- Bring up the volume to 1 L with ddH_2O
- Sterilize by autoclaving for 30 min

To make low-salt LB-agar, add 15 g of agar prior to autoclaving

SOC:
For 1 L
- 20 g tryptone
- 5 g yeast extract
- 0.5 g NaCl
- 0.186 g KCl
- 0.952 g $MgCl_2$
- Bring up the volume to around 900 mL with ddH_2O, then adjust the pH to 7.4 with 10 *N* NaOH
- Bring up the volume to 1 L with ddH_2O
- Sterilize by autoclaving for 30 min
- Add 20 mL of sterile 1 M glucose immediately before use

1 M glucose:
For 100 mL
- Dissolve 18 g glucose in 100 mL ddH_2O
- Sterilize by filtering through 0.2 μ or by autoclaving for 15 min

Antibiotic stocks

Ampicillin (1000x):
- Dissolve 5 g ampicillin in 25 mL ddH_2O
- Add 25 mL absolute ethanol
- Store at -20 °C

Carbenicillin (1000x for liquid media, 2000x for plates):
- Dissolve 2.5 g carbenicillin in 25 mL ddH_2O
- Add 25 mL absolute ethanol
- Store at -20 °C

Chloramphenicol (1000x):
- Dissolve 1.7 g chloramphenicol in 50 mL absolute ethanol
- Store at -20 °C

Kanamycin (1000x, but 250x for autoinduction media):
- Dissolve 1.25 g ampicillin in 50 mL ddH_2O
- Sterilize by filtering through 0.2 μ
- Store at -20 °C in 1.5-mL aliquots

Tetracycline (1000x):
- Dissolve 0.75 g tetracycline in 50 mL absolute ethanol or isopropanol
- Store at -20 °C in 1.5-mL aliquots