

## Buffer recipes

### **Agarose gel**

0.5 g agarose in 50 mL of 1X TAE (final concentration of agarose 1% w/v)

Heat on hot plate until rolling boil, let cool for 10 minutes

Add ethidium bromide to a final concentration of 0.5 µg/mL before pouring gel

### **Ampicillin 1000X**

5.0 g ampicillin in H<sub>2</sub>O, 50 mL final volume (100 mg/mL final concentration)

Filter through 0.22 µm filter

Make 1 mL aliquots, store at -20°C

Dilute to 100 µg/mL in LB for bacterial cultures

### **APS (ammonium persulfate)**

5 g APS in H<sub>2</sub>O, 50 mL final volume (10% w/v final concentration)

Make aliquots, store at -20°C

### **DTT (dithiothreitol)**

3.86 g of DTT in H<sub>2</sub>O, 25 mL final volume (1 M final concentration)

Filter through 0.22 µm filter

Make 1 mL aliquots, store at -20°C

### **IPTG (isopropyl β-D-1-thiogalactopyranoside)**

5.96 g of IPTG in H<sub>2</sub>O, 25 mL final volume (1 M final concentration)

Filter through 0.22 µm filter

Make 1 mL aliquots, store at -20°C

### **Kanamycin 1000X**

1.25 g of kanamycin in H<sub>2</sub>O, 25 mL final volume (50 mg/mL final concentration)

Filter through 0.22 µm filter

Make 1 mL aliquots, store at -20°C

Dilute to 50 µg/mL in LB for bacterial cultures

### **LB (Luria broth) media**

10 g Bacto-tryptone

5 g yeast extract

10 g NaCl

Add 1 L H<sub>2</sub>O

Autoclave

### **10X PBS (1 L)**

1.4 M NaCl (81.8 g)

270 mM KCl (20.1 g)

100 mM Na<sub>2</sub>HPO<sub>4</sub> (14.2 g)

18 mM KH<sub>2</sub>PO<sub>4</sub> (2.45 g)

pH 8.0

### **PMSF (phenylmethanesulfonyl fluoride)**

0.87 g in 2-propanol (isopropanol), 50 mL final volume (100 mM final concentration)

Filter through 0.22 µm filter

Make 5 mL aliquots, store at -20°C

Warm and vortex to make all dissolve, dilute to 1 mM in cell suspension

**SDS-PAGE destaining solution**

300 mL methanol (30%)  
100 mL acetic acid (10%)  
600 mL H<sub>2</sub>O

**SDS-PAGE gel making buffer**

1.5 M Tris-HCl (for separating gel)  
118.2 g of Tris-HCl in H<sub>2</sub>O, pH 8.8  
Final volume 500 mL  
Filter and degas

**SDS-PAGE gel making buffer**

1 M Tris-HCl (for stacking gel)  
78.8 g of Tris-HCl in H<sub>2</sub>O, pH 6.8  
Final volume 500 mL  
Filter and degas

**SDS-PAGE 10X gel running buffer**

248 mM Trisma (60 g)  
1.92 M glycine (288 g)  
1% w/v SDS (20 g)  
Final volume 2 L  
No need to pH, filter, or degas  
Dilute to 1X for running SDS-PAGE gels

**SDS-PAGE marker buffer**

4.8 mL of H<sub>2</sub>O  
1.2 mL of 1 M Tris-HCl pH 6.8  
1 mL of 100% glycerol  
2 mL of 10% w/v SDS (sodium dodecyl sulfate)  
0.5 mL of 0.1% w/v bromophenol blue

**SDS-PAGE marker**

25 µL of marker (Bio-Rad catalog number 161-0317)  
25 µL of 2-mercaptoethanol (BME)  
450 µL of SDS-PAGE marker buffer  
Heat at 95°C for 5 minutes and store at -20°C

**4X SDS-PAGE sample loading buffer**

1.5 mL of 1 M Tris-HCl pH 6.8  
3 mL of 1 M DTT (dithiothreitol)  
0.6 g of SDS (sodium dodecyl sulfate)  
0.03 g of bromophenol blue  
2.4 mL of glycerol  
Bring final volume to 7.5 mL  
If solution is orange/yellow in color, add 1 drop of 5 M NaOH to adjust pH  
Make 500 µL aliquots and store at -20°C

**SDS-PAGE Coomassie staining solution**

1.25 g Coomassie R-250  
225 mL methanol  
225 mL H<sub>2</sub>O  
50 mL glacial acetic acid

**50X TAE buffer for agarose gels**

242 g Trisma

20.81 g EDTA

57.1 mL glacial acetic acid

Does not need to be pH adjusted

Final volume 1 L