Electrophoresis Buffers And Solutions

NOTE: Use ACS or better grade chemicals, and ddH₂O for all buffers.

**SDS-PAGE Gels**

**5X Resolving Buffer**

500 mls

- Tris-HCl (Sigma) 27.5 g
- Tris-Base (Sigma) 100 g
- SDS (BioRad) 2.5 g

Filter through 0.45 µm.

**5X Stacking Buffer**

500 mls

- Tris-HCl (Sigma) 38.5 g
- Tris-Base (Sigma) 1.5 g
- SDS (BioRad) 2.5 g

Filter through 0.45 µm.

**30% Acrylamide Solution (For SDS-PAGE): 200 mls**

- 60 g Acrylamide
- 1.6 g Methylene Bis acrylamide (1:37.5)

Bring to 200 ml with ddH₂O

Filter through 0.45 µm. Store at 4°C.

Exposure to light as little as possible.

**SDS PAGE Sample Buffer**

9/10 volume SB1 or SB2 or SB3

1/10 volume 1M DTT

1/100 volume Bromophenol Blue (BPB)

Dilute at least 1 volume sample with 4 volumes sample buffer.

**Sample Buffer 1 (SB1) (For most samples)**

- 2 g glycerol
- 0.4 g SDS (BioRad)
- 0.1 g Tris Base (Sigma) (83 mM)
- 0.2 g Tris-HCl (Sigma) (127 mM)

ddH₂O to 9 ml. pH ~8

**Sample Buffer 2 (SB2) (For TCA precipitates)**

- 2 g glycerol
- 0.4 g SDS (BioRad)
- 0.3 g Tris Base (Sigma) (248 mM)

ddH₂O to 9 ml. pH ~10
**Bromophenol Blue (BPB), 100X**

0.2 g BPB dye, ACS Free Acid  
0.2 g Tris Base  
Bring volume to 10 mls with ddH$_2$O.

**10 X Reservoir Buffer**  
1 L of 1X Ultrapure  
288 g Glycine  
60 g Tris-Base  
20 g SDS (BioRad)  
Bring to 2 liters with ddH$_2$O.  
10 µl ThioGlycolate

**SDS PAGE Gel Fixer (2X)**

800 mls MeOH  
200 mls AcCOOH  
Dilute 1:1 with dH$_2$O prior to use.

**SDS PAGE Gel Stain**

450 mls MeOH  
100 mls AcCOOH  
450 mls ddH$_2$O  
1.0 g Coomassie Blue dye. Mix overnight before use.

**SDS PAGE Gel Destain (5X)**

500 mls MeOH  
500 mls AcCOOH  
Dilute 1:4 with dH$_2$O prior to use.

**Western Transfers**

**10X Semi-Dry (BS-N) Western Transfer Buffer**

58 g Tris Base (pH ~9.2)  
29 g Glycine  
2 g SDS  
Bring volume to 1000 mls with ddH$_2$O.

**10X Towbin Western Transfer Buffer**

144 g Glycine (pH ~8.3)  
30 g Tris Base  
2 g SDS  
Bring volume to 1000 mls with ddH$_2$O.
Urea Polyacrylamide Gels

10X TBE: 500 mls
54 g Tris Base
27.5 g Boric Acid
3.72 g Na$_2$EDTA $\cdot$ 2H$_2$O (BRL Ultrapure EDTA)

40% Acrylamide Stock Solution (For Nucleic Acid Gels): 200 mls
76 g Acrylamide
4 g Methylene Bis acrylamide (1:19)
Bring to 200 ml with ddH$_2$O
Store at 4°C. Expose to light as little as possible.

DNA Agarose Gels

50X TAE: 500 mls
121 g Tris Base
9.4 g Na$_2$EDTA $\cdot$ 2H$_2$O (BRL Ultrapure EDTA)
Stir Tris and EDTA into solution. Add 30 mls glacial AcCOOH.
Adjust pH to 8.0 with additional glacial AcCOOH (~5 ml)

10X DNA Loading Solution: 10 mls
2 g Ficoll 400
1 g Glycerol (ACS grade)
2 ml 0.5 M EDTA, pH 8
1 ml 10% SDS
100 µl Bromphenol Blue (100X)
Add ddH$_2$O to 10 mls. Mix completely (rotator).
Divide into 1 ml aliquots. Store at 4°C.

Formaldehyde RNA Gels

10X MOPS: 500 mls
41.9 g MOPS 400 mM MOPS
6.80 g NaOAc 100 mM NaOAc
1.86 g Na$_2$EDTA 10 mM EDTA
Adjust to pH 7.0 with NaOH pellets and 5N NaOH
Treat with DEPC and autoclave (will yellow)

Loading Buffer:
50% Glycerol
1 mM EDTA, pH 8
0.25% BPB dye
Treat with DEPC
**Glyoxal RNA Gels**

**Deionized Glyoxal:**
1. Thaw 40% glyoxal stock, warm to 50°C, and transfer 20 mls to a 50 ml plastic tube. Transfer the remainder of the glyoxal stock to 50 ml tubes, parafilm, and immediately freeze at -70°C. Glyoxal can not be stored more than ~1 year at -20°C. Work as quickly as possible: exposure to air slowly degrades glyoxal.
2. Add Biorad AG 501-X8 (D) ion exchange resin. Fill to ~10 ml mark on tube.
3. Mix gently for 5 minutes on end-over-end rotator. If resin turns gold in color, it is exhausted and more resin should be added.
4. Check pH. Should be >5.0. Remove 20 µl with pipettor and check with pH paper (range 4-7). Don't place pH paper in Glyoxal.
5. Pour glyoxal and beads into a syringe with 0.45 µm filter. Gently apply pressure and collect as much glyoxal as possible (~10-15 mls). Prepare 0.5 ml aliquots in 0.5 ml microfuge tubes and store at -70°C.

**Glyoxal/DMSO (G/D) Solution:**
1. Thaw 0.5 ml deionized glyoxal (aliquots at -70°C).
2. Mix with 1.5 ml DMSO (Aldrich HPLC Grade Methyl Sulfoxide).
3. Add 0.3 ml DEPC treated 0.1M NaPi, pH 7.0. Mix quickly, but completely.
4. Quickly aliquot 100 µl volumes in 0.5 ml tubes and freeze.
5. Store at -70°C in pill box.

**Loading Solution for Glyoxal/DMSO samples:**
- 50% glycerol
- 10 mM NaPi, pH 7.0
- 0.25% BPB (0.25% XC optional)
- Treat with DEPC.