

## Schwer Lab

### Some protein extraction, SDS-PAGE, and western blotting buffers:

#### **10X SDS-PAGE Running buffer**

60 g Tris base  
288 g glycine  
20 g SDS  
water to 2 L  
Store at RT.

#### **10X Transfer buffer**

60 g Tris base  
288 g glycine  
water to 2 L  
Store at RT.

#### **1X Transfer buffer [10% methanol]**

200 mL 10X Transfer buffer  
1600 mL water  
200 mL methanol  
Store at 4°C

#### **6× SDS sample buffer**

7 mL 4× Tris-Cl/SDS, pH 6.8 (recipe below)  
3 mL glycerol (30% final)  
1 g SDS [10% final]  
0.93 g DTT (0.6 M final)  
1.2 mg bromophenol blue (0.012% final)  
Add MilliQ water to 10 mL  
Store in 0.5-ml aliquots at -70°C

#### **4X Tris-Cl/SDS, pH 6.8 (0.5 M Tris-Cl containing 0.4% SDS) [stacking gel buffer]**

Dissolve 6.05 g Tris base in 40 mL H<sub>2</sub>O. Adjust to pH 6.8 with 1 N HCl. Add H<sub>2</sub>O to 100 mL total volume. Filter solution through a 0.45-µm filter, add 0.4 g SDS. Store at 4°C for up to 6 months.

#### **4X Tris-Cl/SDS, pH 8.8 (1.5 M Tris-Cl containing 0.4% SDS) [gel buffer]**

Dissolve 91 g Tris base in 300 mL H<sub>2</sub>O. Adjust to pH 8.8 with 1 N HCl. Add H<sub>2</sub>O to 500 mL total volume. Filter the solution through a 0.45-µm filter, add 2 g SDS. Store at 4°C for up to 6 months.

#### **2 Liter 10× TBS:**

48.4 g Tris base  
160 g NaCl  
Adjust pH to 7.6-8.0 with HCl (~19 mL concentrated HCl)

#### **1× TBS-T**

200 mL 10× TBS  
20 mL 10% (w/v) Tween-20  
Water to 2 L.  
Store at RT.

**Western blocking solution**

5% (w/v) non-fat dry milk in 1X TBS-T,

**Antibody dilution solution**

Use western blocking solution or 5% BSA in 1×TBS-T.