**PhosTag / Western Blot / Broad range Buffers**

**30% (w/v)** **Acrylamide Solution (30% T, 3.3% C) (unless purchased)**

1. Combine
   1. 29.0 g of acrylamide
   2. 1.0 g of N,N'-methylene-bisacrylamide
2. Make to 100 mL with distilled water. Filter and store at 4˚C in the dark.

**1.5 mol/L** **Tris/HCl Solution, pH 8.8 (4x solution for resolving gel)**

1. Add 40 mL of DI water to a beaker and place on ‘Ramsey’ with a stir of 250 RPM and a temp 30 degrees C
2. Add in;
   1. 18.2 g of Tris base (FW: 121, pKa = 8.2 at 20˚C)
3. Add HCl to bring solution to a pH of 8.8
4. Fill to 100 mL with distilled water. Store at Room temp.

**0.50 mol/L** **Tris/HCl Solution, pH 6.8 (4x solution for stacking gel)**

1. Add 40 mL of DI water to a beaker and place on ‘Ramsey’ with a stir of 250 RPM and a temp 30 degrees C
2. Add in;
   1. 6.06 g of Tris base (FW: 121, pKa = 8.2 at 20˚C)
3. Add HCl to bring solution to a pH of 6.0
4. Fill to 100 mL with distilled water. Store at Room temp.

**10% (w/v)** **SDS Solution**

1. Dissolve 10.0 g SDS in 90 mL of distilled water with stirring
2. Bring to total volume to 100 mL with distilled water. Store at Room temp.

**5.0 mmol/L** **Phos-tagTM AAL Solution containing 3% (v/v) MeOH**

1. In a plastic tube, dissolve 2 mg of Phos-tagTM AAL-107 into 20 µL of methanol
2. Add in 640 µL distilled water

The viscous oil product, Phos-tagTM AAL-107 (10 mg) placed in a plastic tube is completely dissolved in 0.10 mL methanol. The solution is diluted with 3.2 mL distilled water by pipetting.

*Note: If a trace amount of insoluble material appeared as white fine powder (impurity) in the solution, it can be separated by centrifuging using two 2-mL microtubes.*

*Store the solution in the 2-mL microtubes at 4˚C in the dark. From the supernatant solution,*

*45 mini-slab gels (50 µmol/L Phos-tagTM, 1-mm-thick, 9-cm-wide, 7.7-cm-long) can be prepared.*

**10 mmol/L** **MnCl2 Solution**

1. Dissolve 64 mg MnCl2 in 50 mL of distilled water.

Note: Do not use the other anion salt, such as Mn(NO3)2 and Mn(CH3COO)2.

**10% (w/v)** **Ammonium Persulfate Solution (10% APS)**

1. Dissolve 10 mg (NH4)2S2O8 (FW: 228) in 0.10 mL of distilled water.

Note: Freshly prepare prior to use.

**Running Buffer, pH 8.3 (10x solution)**

1. Dissolve;
   1. 15.1 g of Tris base (0.25 mol/L)
   2. 5.0 g of SDS
   3. 72.0 g of glycine (1.92 mol/L)
2. Make to 0.50 L with distilled water. Do not adjust pH with acid or base. Store at 4˚C.

Use: Dilute 50 mL of the 10x solution with 450 mL distilled water.

OR use the pre brought concentrate;

1. Dissolve;
   1. Fill beaker with roughly 200 mL of distilled water
   2. Add 50 mL of Tris/SDS 10x
2. Fill to 500 mL distilled water and store at 4˚C.

**Sample Buffer (3x solution)**

1. Dissolve;
   1. 1.5 mg Bromophenol Blue (BPB, a tracking dye)
   2. 0.60 g SDS
   3. 3.0 mL glycerol
   4. 3.9 mL 0.50 mol/L Tris/HCl, pH 6.8
   5. 1.5 mL 2-mercaptoethanol
2. Make to 10 mL with distilled water. Store at –20˚C.

**Acidic Solution for Fixation of Proteins (1 L) for 30-60 minutes**

1. Combine;
   1. 0.10 L of acetic acid
   2. 0.50 L of methanol
   3. 0.40 L of distilled water

**Coomassie Staining Solution (CBB) (0.5 L) for 2-4 hours (or overnight)**

1. Dissolve;
   1. 0.25 g of Coomassie Brilliant Blue (CBB)
   2. 40 mL of methanol
2. Then add;
   1. 10 mL of acetic acid
   2. 50 mL of distilled water

**Washing and De-staining Solution (1 L) until desired background is achieved**

1. Combine;
   1. 10 mL of acetic acid
   2. 50 mL of methanol
   3. 40 mL of distilled water

**Resolving Gel Solution (10 mL: 12% (w/v) acrylamide & 50 µmol/L Phos-tagTM AAL)**

1. Combine:
   1. 4.00 mL 30% (w/v) Acrylamide Solution
   2. 2.50 mL 1.5 mol/L Tris/HCl Solution, pH 8.8
   3. 100 µL 5.0 mmol/L Phos-tagTM AAL Solution
   4. 100 µL 10 mmol/L MnCl2 Solution
   5. 100 µL 10% (w/v) SDS Solution
   6. 3.15 mL Distilled Water
   7. 50 µL 10% (w/v) Ammonium Persulfate (APS)
2. Lastly, add 8 µL of TEMED (tetramethylethylenediamine)

**Stacking Gel Solution (e.g., 4.5% (w/v) acrylamide) total 4 mL**

1. Combine:
   1. 600 µL 30% (w/v) Acrylamide Solution
   2. 100 µL 0.50 mol/L Tris/HCl Solution, pH 6.8
   3. 100 µL 10% (w/v) SDS Solution
   4. 2.34 mL Distilled Water
   5. 20 µL 10% (w/v) Ammonium Persulfate (APS)
2. Lastly, add 8 µL of TEMED (tetramethylethylenediamine)

**Mn2+ – Phos-tagTM SDS-PAGE adopts almost the same gel compositions for Laemmli's method, but SDS may be unnecessary as an additive in the resolving and stacking gels.** **In the presence of SDS in the gel, the band of target protein would be rather broad and/or tailing.**

**Most pre-stained molecular weight markers contain**

* 1. **mM EDTA**

**You can add 1 mM MnCl2 to markers to complex the free EDTA**

**Transfer Buffer (for each blot I would make 2 Liters)**

1. In 500 ml of Di water, Combine:
   1. 150 mL Methanol
   2. 40 mL Tris-Glycine Buffer (in box above bench)
   3. 1.3 g SDS
2. Fill to 1 L with distilled water.
3. Remove 100 mL of the buffer and add 0.2922 grams of EDTA to it to make **Transfer Buffer + 10 mM EDTA**