Recipes  
PARAM LAB  
  
Western-Blot Grade Reagents:

**1.5M Tris pH 8.80 (250mL)**

**\*** Calibrate pH meter **\* \*** Stable at RT for 6 months **\***

1. Weigh 45.43g of Tris Base and dissolve it in 190mL of ddH20
2. Stir until solution is homogenous
3. Add concentrated acid (HCl) drop-wise until desired pH is reached. Record volume of acid used
4. Add ddH20 to reach the desired final volume
5. Autoclave

**0.5M Tris pH 6.8 (250mL)**

**\*** Calibrate pH meter **\* \*** Stable at RT for 6 months **\***

1. Weigh 15.14g of Tris Base and dissolve it in 200mL of ddH20
2. Stir until solution is homogenous
3. Add concentrated acid (HCl) drop-wise until desired pH is reached. Record volume of acid used
4. Add ddH20 to reach the desired final volume
5. Autoclave

**1M Tris pH 6.8 (250mL)**

**\*** Calibrate pH meter **\* \*** Stable at RT for 6 months **\***

1. Weigh 30.29g of Tris Base and dissolve it in 190mL of ddH20
2. Stir until solution is homogenous
3. Add concentrated acid (HCl) drop-wise until desired pH is reached. Record volume of acid used
4. Add ddH20 to reach the desired final volume
5. Autoclave

**1M Tris pH 6.8 (250mL)**

**\*** Calibrate pH meter **\* \*** Stable at RT for 6 months **\***

1. Weigh 30.29g of Tris Base and dissolve it in 190mL of ddH20
2. Stir until solution is homogenous
3. Add concentrated acid (HCl) drop-wise until desired pH is reached. Record volume of acid used
4. Add ddH20 to reach the desired final volume
5. Autoclave

**30% Acrylamide/bis-Acrylamide (100mL)**

**\*** Stable at 4°C for 6 months **\***

1. Weigh 29g of Acrylamide and 1g of N,N'-methylbisacrylamide
2. Add these reagents to 60mL of ddH20
3. Stir until solution is homogenous
4. Add 40ml of ddH20
5. Filter through a 0.45µm filter pore

**48% Acrylamie/ bis-Acrylamide (100mL)**

**\*** Stable at 4°C for 6 months **\***

1. Weigh 47g of Acrylamide and 1g of N,N'-methylbisacrylamide
2. Add these reagents to 60mL of ddH20
3. Stir until solution is homogenous
4. Add 40ml of ddH20
5. Filter through a 0.45µm filter pore

**10% APS (5mL)**

**\*** Needs Autoclaved ddH20 **\* \*** Six Month Expiration Date \*

1. Weigh 0.5mg of APS
2. Add to a 15mL conical tube containing 5mL autoclaved ddH20
3. Vortex until solution is homogenous
4. Aliquot into micro-centrifuge tubes by 250uL partitions
5. Label aliquots and place in vial box in -20°C. Label with expiration date
6. Each aliquot is meant for single use. Throw away after a single freeze-thaw cycle

**10% SDS (250mL)**

**\*** Stable at RT for 6 months **\***

1. Weigh 10mg of SDS
2. Dissolve the SDS in 80mL of ddH20
3. Stir until solution is homogenous
4. Add 20mL of ddH20
5. Label with expiration date

**1% and 0.5% Bromophenol Blue (10mL)**

**\*** Stable at RT for 6 months **\***

1. Weigh 0.1g of BPB and dissolve in 10mL ddH20
2. Vortex thoroughly until solution is homogenous
3. **This makes 10mL of 1% BPB**
4. Add 5mL of 1% BFB to 5mL of ddH20
5. Vortex frequently until homogeneous
6. **This makes 10mL of 0.5% BPB and 5mL of 1% BPB**

**Laemmli (SDS-Reducing) Sample Buffer (9.5mL)**

**\*** Stable at RT for 6 months **\***

1. Add these reagents in order in a 15mL conical tube to make stock solution

|  |  |
| --- | --- |
| ddH20 | 3.55mL |
| 0.5M Tris-HCl, pH 6.8 | 1.25mL |
| **Glycerol\*** | 2.5mL |
| 10% SDS | 0.2mL |
| 0.5% Bromophenol Blue | 0.2mL |
| Total Volume | 9.5mL |

**\*Glycerol** should be reverse-pipetted with small portion of pipette tip snipped

1. Add 50uL of β-mercaptoethanol to 950uL sample buffer to make working solution prior to loading gel.

**20X TBS (100mL)**

\* Store at RT \* \* Calibrate pH meter \*

1. Weigh 17.54 of NaCl and 4.8g of Tris Base, and dissolve in 80mL ddH20
2. Stir until solution is homogenous
3. Adjust pH to 7.6 with concentrated HCl and record volume of acid used
4. Add remaining volume to bring up to 100mL

**1X TBS-T (1000mL)**

\* Store at RT \* \* 6-month expiration date \*

1. Add 50mL of 20X TBS to 900mL ddH20
2. Add 1mL of Tween 20 by reverse-pipetting and snipping tip
3. Add 50mL of ddH20

**1X Running Buffer (4L)**

\* Store in 4°C \*

1. Weigh 57.7g of glycine and 12.1g Tris Base, and 4g SDS and dissolve in 1900mL ddH20
2. Stir until solution is homogenous
3. Add solution to 4L bottle along with 2100mL ddH20
4. Invert bottle

**1X Transfer Buffer (4L)**

\* Store in 4°C \*

1. Weigh 57.7g of glycine and 12.1g Tris Base and dissolve in 1900mL ddH20
2. Stir until solution is homogenous
3. Add 600mL (474.75g on scale) of methanol to a 4L bottle
4. Add glycine and tris solution to 4L bottle along with 600mL ddH20
5. Invert bottle