GATHER REAGENTS & MATERIALS
30% acrylamide/0.8% bisacrylamide (30:1)
4X Tris.Cl/SDS, pH 8.8
4X Tris.Cl/SDS, pH 6.8
Ammonium persulfate, 10% (Make fresh each time.)
SDS electrophoresis buffer, 1X (5X stock available)
Temed
Minigel vertical gel unit with glass plates, clamps, and buffer chambers 0.75-mm spacers
multiple gel caster
pipettors w/tips (P1000, P100, and P10)
aspirator
Assemble multiple gel caster (4 gels)

SEPARATING GEL
1. In a 50 ml conical tube, add 7.5 mls 4X Tris.Cl/SDS, pH 8.8
2. Add 30% acrylamide/0.8% bisacrylamide
   - 8% gel = 8 mls
   - 10% gel = 10 mls**
   - 12% gel = 12 mls
3. Bring to 30 mls with dH2O.
4. Degas under vacuum 15 minutes.
5. Add 100 uls 10% APS and 20 uls TEMED. Invert tube gently to mix.
6. Pour into multicaster.
7. Overlay each gel of the 4 individual gels with 200 uls saturated isobutanol.
8. Polymerize for 60 minutes.

STACKING GEL (4.0% acrylamide)
1. In a 50 ml conical tube, add 3.75 mls 4X Tris.Cl/SDS, pH 6.8
2. Add 2.0 mls 30% acrylamide/0.8% bisacrylamide
3. Bring to 15 mls with dH2O.
4. Degas under vacuum 15 minutes.
5. Wash isobutanol off separating gels with dH2O. Remove excess water.
6. Add 50 uls 10% APS and 10 uls TEMED to gel solution. Swirl gently to mix.
7. Overlay each gel with stacking gel solution.
8. Insert combs and allow to polymerize for 60 minutes.

Gels may be stored for 1 week in 1x Separating gel buffer.
Recipes:

**4X Tris Cl/SDS, pH 8.8**
Dissolve 45.5 g Tris base (1.5 M) in 150 mls H2O. Adjust pH to 8.8 with 1N HCl. Add H2O to 250 ml total volume. Filter solution through a 0.45 um filter, add 1 g SDS [0.4% (w/v)], store at 4°C up to 1 month.

**4X Tris Cl/SDS, pH 6.8**
Dissolve 6.05 g Tris base (0.5 M) in 40 mls H2O. Adjust pH to 6.8 with 1N HCl. Add H2O to 100 ml total volume. Filter solution through a 0.45 um filter, add 0.4 g SDS [0.4% (w/v)], store at 4°C up to 1 month.

**Ammonium persulfate, 10%**
100 mg ammonium persulfate in 1 ml H2O. Make fresh each time.

**SDS electrophoresis buffer, 5 X**
15.1 g Tris base (0.125 M)
72.0 g glycine (0.96 M)
5.0 g SDS [0.5 (w/v)]
H2O to 1000 ml
Dilute to 1X before use.
Do not adjust the pH of the stock solution, as the pH is 8.3 when diluted. Store at 0° to 4°C until use.

**SDS sample buffer, 6X**
7 ml 4X Tris.Cl/SDS, pH 6.8
3.0 ml glycerol [30% (v/v)]
1 g SDS [1% (w/v)]
0.93 g DTT (0.5 M)
1.2 mg bromphenol blue [0.0012% (w/v)]
Add H2O to 10 ml if needed
Store in 0.5 ml aliquots at -70°C.

**Coomassie Blue Stain**
Methanol 50% (v/v) 500 mls
Coomassie brilliant blue R-250 0.05% 0.5 g
Acetic acid 40% (v/v) 400 mls
dH2O 100 mls

**Destain**
Methanol 250 ml
Acetic acid 100 ml
dH2O 650 ml
<table>
<thead>
<tr>
<th>Transfer buffer (PVDF membrane)</th>
<th>Nitrocellulose membrane</th>
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<tbody>
<tr>
<td>Tris base</td>
<td>18.2</td>
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<tr>
<td>Glycine</td>
<td>86.5</td>
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<tr>
<td>dH2O</td>
<td>4,000 ml</td>
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<tr>
<td>15% MeOH</td>
<td>900 mls</td>
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<tr>
<td>dH2O</td>
<td>qs 6,000 mls</td>
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<td>10% MeOH (600 mls)</td>
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**Tris-Buffered Saline (TBS) pH 7.4**

100 mM Tris.Cl pH 7.5 (0.1M)(121.1)(4) = 48.4 g  
0.9% NaCl (0.9 g/100 mls) = 40.0 g  
dH2O = 4 liters

**TTBS (0.5% Tween ~20)**  
Add .5 mls Tween-20/ 100 mls or 5 mls/liter

**Blocking Buffer**  
TTBS w/ 10% carnation milk (10 g/100 mls TTBS)
**SAMPLE PREPARATION**

1. Determine the protein concentration.
2. Add 6X sample buffer (1 µl of 6X SB/5 µl sample)
3. Determine final protein concentration of sample = Original concentration x 0.833.
4. Denature protein in boiling water bath
5. Record the amount of protein loaded/lane. Typically 30-50 µg/lane.
   For some applications, for example zebrafish embryos, lanes may be loaded on a per embryo basis instead of a particular protein concentration.
6. Run the gel at 200 volts until the dye front reaches the end of the gel (~ 40 minutes)

**Record the following information in your lab books**

GEL #: _________.
DATE:___________.
% ACRYLAMIDE ___________.

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<tr>
<th>Lane</th>
<th>Extract ID</th>
<th>amt. Protein</th>
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**FOR WESTERNS AND SOUTHWESTERNS:**

BUFFER USED:__________________________________________________.
BLOCKING AGENT:__________________________________________________.
PRIMARY Ab.(or OLIGO):_________ DILUTION:_________.
SECONDARY Ab (or OLIGO):_________ DILUTION:_________.
DEVELOPMENT:__________________________________________________.
RESULTS:______________________________________________________.
WESTERN BLOATING

1. Cut PVDF membrane to cover gel.
2. Wet membrane in 100% MeOH for 30 seconds, rinse in dH₂O for 3 minutes, then soak in transfer buffer for 5 minutes.
3. Assemble transfer apparatus as follows (all materials must be saturated with transfer buffer):
   - Black grid down
   - Scotch bright pad
   - Filter paper
   - Gel
   - Membrane
   - Filter paper
   - Scotch bright pad
   - White grid on top
4. Insert so that white grid faces the red electrode, run for 1 hour at 100 Volts.
5. Remove membrane, fix in 100% MeOH for 1 minute (Membrane can be air-dried and stored at 4°C if needed).
6. Wash fixed membrane in small amount of blocking buffer for several minutes, then incubate in fresh blocking buffer for at least one hour.

7. Add primary antibody diluted at the recommended ratio in Blocking buffer.
8. Incubate overnight at 4°C with shaking (Place a stir-plate in the refrigerator in room 208).
9. Remove primary antibody, wash with TTBS 3 X 15’
10 Add secondary antibody diluted at the recommended ratio in TTBS.
11. Incubate for at least 1 hour at room temperature.
12. Remove secondary antibody, wash with TTBS 3 X 15’