**Gel Electrophoresis**

1. Thaw samples on the ice. Turn on the heater at 95°C.

2. Make gel with cassette (invitrogen) and prepare samples.

   Separating gel: 7.5% polymerase gel with 4% shocker.

   - ddH2O: 4.14 ml
   - Tris HCL PH=8.8: 1.875 ml
   - 20% SDS: 37.5 μl
   - A/Bis: 1.405 ml
   - Mix together and then add:
     - 10% APS: 37.5 μl
     - TEMED: 5 μl

3. Pipette the gel into cassette until it reaches 2nd line.

4. Add water sol. Bustinol (not too much) to prevent evaporation.

5. Wait 45-60 min until the gel has fully polymerized

6. Retrieve: tissue, lysis buffer (stored at -20°C), loading dye (stored at -20°C)

   - Volume: 25 μl, Protein: 30-50 μg
   - Then, add 1/20 volume β-ME
   - Centrifuge for 1min, heat 5 min or more at 95°C, store at RT until use.

7. When gel has polymerized, discard the water sol. Bustinol, rinse thoroughly with ddH2O, wipe off.

8. Prepare loading gel: 4% polymerase gel.

   - ddH2O: 1.92 ml (RT)
   - Tris HCL: 0.75 ml (RT) PH=6.8
   - 20% SDS: 15 μl (RT)
   - A/Bis: 0.3 ml (4°C)
   - Mix then add:
     - 10% APS: 5 μl (-20°C)
     - TEMED: 2 μl (RT)

9. Pipette into gel cassette, insert comb, and wait approx. 1hr

10. Mark bottom of wells with a marker, remove the comb and rinse thoroughly.

11. Place the gels into electrophoresis apparatus

12. Load 1* SDS Elution buffer (Dilute 10x stock 1:10 diluted in ddH2O) between the gels until it reaches the first line.

13. Load the marker (ladder) and samples.

14. Load 1* SDS Elution buffer outside the gels until the samples are immersed

15. Run the gel 75V 5~10 min until blue dye pass the first line

16. Pause apparatus and turn voltage to 120 V for 1.5 hr -> 2.5 hr until blue dye is approximately 0.5-1 mm above the bottom.

   Note: if the blue dye is not straight, the gel did not perfectly polymerase
Transfer the protein to membrane
1. Pre-wet the sponge and filter paper, membrane with \( \frac{1}{2} \) * Transfer buffer stock.
   \( \frac{1}{2} \) * Transfer buffer
   - 10* transfer buffer 50 ml
   - Methanon (RT Hood) 50 ml
   - 900 ml ddH₂O Stored at 4°C

2. Wet transfer membranes with ddH₂O (for nitrocellulose membranes) and then soak transfer membranes in transfer buffer stock for several minutes.
3. Remove gels from cassette and create the transfer sandwich as follows
   
   Top
   ------------------------- Sponge *2 (blotting pad)
   ------------------------- Filter paper
   ------------------------- Transfer membrane
   ------------------------- Gel
   ------------------------- Filter paper
   
   Bottom
   ------------------------- Sponge *2 (blotting pad)

   Put a sponge between them if transferring two membranes at the same time.
4. And run overnight at 15 V in cold room.

Western blot
1. Remove the membrane, rinse with TBST (Tris –buffered Tween-20)
   
   Recipe: TBST
   - 10X TBS 100 ml
   - ddH₂O 900 ml
   - tween-20 500 µl (hood)

2. Wash membrane in TBST (3x15 minutes)
3. Incubate membrane in TBSTM 1 hour to block.
   
   Recipe: Tris buffered saline tween -20 w milk
   - dry milk 10 g
   - 10X TBS 20 ml
   - Tween- 20 100 µl
   - dd H₂O 180 ml

4. Incubate in primary antibody in TBSTM for 2 hour
5. Wash membrane in TBST (3x15 minutes)
6. Incubate in secondary antibody 1 hours (usually use 2° antibody at 1:4000)
7. Wash membrane with TBST (3x15 minutes)
8. Turn on film developer (standby), prepare ECL reagent (1ml Solution A, 1ml Solution B for each membrane)
10. In darkroom, place film on membrane for 2 min – 5 min, and then place film in processor.
11. Membranes can then be discarded or stripped of antibody and then reprobed.
12. For stripping, rinse membrane (3 X10min) with TBST.
Incubate in stripping buffer for 30min-1hr at room temperature.

Rinse (3X10 min) in TBST. Repeat western procedure.

Recipe: 10 * SDS elution buffer stock

- Tris base (0.25M) 30.3 g
- Glycine (1.92M) 144 g
- SDS 1.0 g
- Dilute to 1000 ml with ddH2O
- Stored at 4 °C

Recipe: 10X transfer buffer

- Tris Base 30.3 g (0.25 M)
- Glycine 144 g (1.92 M)
- Diluted to 1000 ml with ddH2O PH=8.2-8.3. stored at 4°C

Recipe: 10X TBS (Tris buffered saline)

- Trizma 24.2 g (0.2m)
- Sodium chloride 80 g (1.37M)
- PH to 7.6 using 6M HCL
- Diluted to 1000 ml with ddH2O stored at RT

Recipe: Stripping buffer

**Medium stripping buffer:**

- 15 g glycine
- 1 g SDS
- 10 ml Tween20
- Set the pH to 2.2
- make up to 1 L with ultrapure water

**Harsh stripping buffer:** to be done under the fumehood

For 100 ml:

- 20 ml SDS 10%
- 12.5 ml Tris HCl pH 6.8 0.5M
- 67.5 ml ultra pure water
- Add 0.8ml β-mercaptoethanol under the fumehood.