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.

ddH_2O	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 ddH_2O, wipe off.

8. Prepare loading gel: 4% polymerase gel.

ddH_2O 1.92 ml (RT)	
Tris HCL	0.75 ml (RT) PH=6.8
20% SDS	15 μl (RT)
A/Bis	0.3 ml (4^oC)
Mix then add:	
10% APS 1	5 μl (-20^oC)
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 ddH_2O) 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 ½ * Transfer buffer stock.

½ * Transfer buffer

10* transfer buffer	50 ml
Methanon (RT Hood)	50 ml
900 ml ddH_2O Stored at 4°C	

2. Wet transfer membranes with ddH_2O (for nitrocellulose membranes) and then soak transfer membranes is 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
ddH_2O	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^o 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)

9. Discard TBST, and pipette ECL solution onto membrane. Let stand 2 minutes.

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.

13. Incubate in stripping buffer for 30min-1hr at room temperature.

14. 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 ddH_2O
Stored at 4 °C

Recipe: 10 X transfer buffer

Tris Base	30.3 g (0.25 M)
Glycine	144 g (1.92 M)

Diluted to 1000 ml with ddH_2O 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 ddH_2O 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 ß-mercaptoethanol under the fumehood.