Membrane preparation

1. Gel preparation

Prepare 14% separation gel. (Please see the following tables for the components)

Stacking gel: 4%

	2.2 ml
H_2O	1.32 ml
0.5M Tris-HCl PH:6.8	0.55 ml
30% Acr/Bis	0.295 ml
10% SDS	22 µl
10% APS	11 µl
TEMED	2.2 µl

Separation gel: 14%

H_2O	2.1 ml
1.5M Tris-HCl PH:8.8	2.0 ml
30% Acr/Bis	3.74 ml
10% SDS	80 µl
10% APS	80 µl
TEMED	3.5 µl

2. Sample loading

Boil the lysates for 5 minutes. Load 10 or 20 µg onto SDS-PAGE gel per lane.

3. Electrophoresis

150V for 1 hour in pre-cold electrophoresis buffer.

4. Transfer stand by

1) After electrophoresis, immerse the gel in pre-cold 1 x electrical transfer buffer before electrical transfer for 10 minutes.

2) Activate PVDF (0.45 µm) membrane with 99.5% methanol for 15 seconds. Wash the membranes with deionized water for 3 times.

3) Immerse PVDF membrane, filter paper and sponge in 1 x electrical transfer buffer for 30 minutes before electrical transfer.

5. Transfer (wet method)

72V for 1 hour at cold condition in pre-cold electrical transfer buffer.

6. Wash

Wash the membrane with deionized water twice on shaking table, 10 minutes per time.

7. Storage

Dry the membrane. Then ready to use. For long term storage, store the membrane at 4°C.

Solution preparation

1. Electrophoresis buffer

10 x electrophoresis buffer: Dissolve 151.425g of Tris, 720.67g of Glycine and 50g (1%) of SDS in 3.5L deionized water, and make the final volume to 5L. Store the buffer at room temperature.

1 x electrophoresis buffer: Dilute the buffer by 10 folds when using.

2. Electrical transfer buffer

10 x transfer buffer: Dissolve 151.425g of Tris and 720.67g of Glycine in 3.5L deionized water, and set the volume to 5L. Store the buffer at room temperature.

1 x transfer buffer: Add 500 ml of 10 x Transfer Buffer and 500 ml of Methanol to 4000ml deionized water.