

Western Blot Protocol

Running Gel (Bottom)

3.67ml H₂O
3.75ml Tris pH 8.8
50ul 20%SDS
2.5ml 29:1 40%Acrylamide
50ul 10%APS
7ul TEMED

Stacker(Top Gel)

3.8ml H₂O
625ul Tris pH 6.8
25ul 20%SDS
500ul 40% Acrylamide (29:1)
50ul 10% APS
5ul TEMED

Running Buffer 1000ml

100ml 10x Tris Glycine
5ml 20%SDS
900ml H₂O

Transfer Buffer 1000ml

200ml MeOH
100ml 10x Tris Glycine
700ml H₂O

Milk Sol'n (500ml)

50ml 10xPBS
5g Dry Milk
1.25ml 20% Tween-20
450ml H₂O

4xSB

1ml Tris pH6.8
5ml 20%SDS
1ml Glycerol
500µl BME
400µl 2%B-Blue
2ml H₂O

Nitroblue tetrazolium chloride (NBT) solution :

50 mg/ml in 70% N',N'-Dimethyl formamide (DMF)

5-Bromo-4-chloro-3-indolylphosphate (BCIP) solution :

20 mg/ml in 100% N',N'-Dimethyl formamide (DMF)

Transfer 1hr @ 100V in Cold Room

Dry 15min- O/N

Wash Blot in ~50ml milk sol'n for ~1hr

Incubate with primary (5ml of milk sol'n) 37°C for 1hr

Wash 10min with milk

Incubate with secondary (B53 for LMP or B176 for HA) 37°C for 1hr

Wash 10min with milk sol'n

Wash with H₂O once

Add stain

50ml Stain

45ml H₂O

333ul BCIP

333ul NBT

5ml 10x AP Buffer

250ul MgCl₂