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Western Blot (tank-blot) + Antibody staining

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1 Works for me dx.doi.org/10.17504/protocols.io.8gdhts6

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MATERIALS TEXT

blotting buffer
SDS-Gel
100 % Ethanol
PDVF membrane

Fresh TBS-BSA
1x TBS-BSA, 100 µl Twin-20 and x µl Antibody
TBST

10x TBS:
24g Tris base
88 g NaCl
Dissolve in 900 ml H2O
Adjust pH= 7.6 (HCl)
Fill to 1 L with H2O
--> store at 4° C

1x TBS-BSA (prepare fresh):
50 ml 1x TBS
1.2 g BSA
--> store in fridge overnight

Tween-20 (50%):
10 ml Tween-20
10 ml water

TBST:
1 L 1x TBS
2 ml Tween-20
--> store at 4° C

1x TBS:
100 ml 10x TBS
900 ml H20
--> store at 4° C

Blotting buffer:
100 ml 10x SDS running buffer
200 ml 100% methanol
700 ml H20

SDS Running buffer (10x):
30 g TRIS
144 g Glycin
10 g SDS

Dissolve in 1 L H2O
--> store at RT

Western blot

1 Create blotting buffer stock containing:
- 100mL 10x SDS running buffer
- 200mL 100% Methanol
- 700mL H20

Fill a tray with blotting buffer

2 Transfer SDS gel to the blotting buffer

3 Assemble blotting sandwich (take it, soak sponge and place on it, soak filter paper and place on it, place inverted SDS gel on top without creating bubbles)

4 Add charged PDVF membrane with forceps (charged in 100 % ethanol and than soaked in buffer)

5 Add soaked Filter and Streak out bubbles

6 Add soaked sponge

7 Press togehter and close container

8 Place in blotting apparatus and add blotting buffer

9 Run for 1 h at 100 V 350-500 mA

Antibody staining

10 Add 40 ml resh TBS-BSA in empty pipet tip box.

11 Place membrane from blottig appartus with forceps into the solution

12 Protein side needs to be up

13 Incubate overnight at 4° C with shaking 20 rpm

14 Discard blocking solution

15 50 ml 1x TBS-BSA, 100 µl Tween-20 and x µl Antibody (dependend on the affinity)

16 Incubate for 1 h with shaking

17 Wash 2 times with TBST quickly , then wash 3 times a 10 min with TBST

18 If you are work with a secondary antibody icubate in the secondary antibody solution and repeat steps 17

Antibody staining

19 Develop using ECL (enhanced chemiluminescence) with HRP