OCT 19, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.8gdhts6

Protocol Citation: Igem Dusseldorf 2019. Western Blot (tank-blot) + Antibody staining. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.8gdhts6>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
 We use this protocol and it's working

Created: Oct 18, 2019

Last Modified: Oct 19, 2019

PROTOCOL integer ID:
 28901

Western Blot (tank-blot) + Antibody staining

Igem Dusseldorf¹

¹Heinrich-Heine Universität Düsseldorf

Igem Dusseldorf

MATERIALS

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 H₂O
--> store at 4° C

Blotting buffer:
100 ml 10x SDS running buffer
200 ml 100% methanol
700 ml H₂O

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 H₂OFill 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 together 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 fresh TBS-BSA in empty pipet tip box.
- 11 Place membrane from blotting apparatus 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 μ l Tween-20 and x μ l Antibody (depend 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