Western Blot (tank-blot) + Antibody staining

Igem Dusseldorf

1 Heinrich-Heine Universität Düsseldorf

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Western blot

1. Create blotting buffer stock containing:
   - 100mL 10x SDS running buffer
   - 200mL 100% Methanol
   - 700mL H2O
   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 then 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 resh 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 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 incubate in the secondary antibody solution and repeat steps 17

**Antibody staining**

19 Develop using ECL (enhanced chemiluminescence) with HRP