Benchling

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Introduction

Western Blotting is a way to visualize the expression of proteins with antibodies

Materials

- DTT
- SDS
- TEMED
- Propanol

Procedure

Preparing Samples

1. add 5xSDS loading dye
2. add 10x DTT
3. cook in the heat block at 90°C for 10 mins

Preparing the Gel

4. Prepare the cast(use 1.5mL thickness)
5. look up the recipe at the work station (we use the 10% gel)
6. instead of adding 8µL TEMED, add 15µL
7. mix the tube by inverting
8. pour with the serological pipette
9. Pour 1mL Propanol
10. Let it solidify for 20 min
11. Pour out propanol
12. Prepare Stacking buffer
13. Pour until it flows out the cast and insert the comb
14. let it solidify

Running the Gel

15. Fill the inner chamber with TE until it overflows and run the gel at 120V for 95 min

Transferring the Gel

16. Soak the acrylamide gel and the thick filter paper in TG
17. Activate the PVDF membrane with methanol and then soak it in TG
18. Build sandwich as indicated below. The sandwich can also be assembled with the translucent side on the bottom, the order must stay the same though. Make sure that there are no air bubbles under the gel, between gel and membrane as well as above the gel.
19. Transfer proteins by applying either 90V for 60-90 min (the resulting current should be 200-300 mA) or 30V o/N in the cold room. Add frozen cool pack to transfer chamber.
20. Take membrane and block either 1h at RT orovernight in the cold room, both with 5% milk in TBS-T
21. Primary Antibody (or HRP conjugated antibody) in 5% milk in TBS-T for 2h at room temperature or o/N in cold room
22. Wash membrane 3-4 times with TBS-T for 10 minutes each

23. Incubate secondary antibody in 5% milkin TBS-T for 1-2h at room temperature
24. Wash membrane 3-4 times with TBS-T for 5 minutes each
25. Wash membrane in TBS for 5 min
26. Imaging: start with standard ECL, re-apply Femto ECL as necessary