Western Blot

2017 Protocols

Purpose:

To identify specific amino acid sequences of a protein, or tag, using fluorescently-tagged antibodies.

Required Materials:

- dH_2O
- 1.5M Tris HCl pH 8.8
- 30% Acrylamide/Bis solution (Bio-Rad)
- 10% SDS
- 10% Ammonium Persulfate in dH_2O
- TEMED
- 2X SDS Gel Running Buffer
- Western Blot Apparatus (Gel dock, glass gel plates, etc.)
- Methanol
- 1X Trans-Blot Turbo Transfer Buffer (Bio-Rad)
- Glacial Acetic Acid
- Coomassie Brilliant Blue R-250
- 95% ethanol
- Trans-Blot Turbo Transfer Machine (Bio-Rad)
- Trans-Blot Turbo RTA Transfer Kit (Bio-Rad)
- Tween
- 1X Tris-Buffered Saline

Procedure:

1. Assemble gel polymerizing station according to manufacturer's protocol.
2. To create a running gel combine:
    a. 2.4 mL dH_2O
    b. 1.5 mL 1.5M Tris HCl pH 8.8
    c. 2 mL 30% Acrylamide/Bis solution (Bio-Rad)
    d. 60 µL 10% SDS
    e. 60 µL 10% APS (must be made fresh every time)
    f. 6 µL TEMED
3. Pour into gel cassette and let polymerize.
4. To create a stacking gel combine:
    a. 6.85 mL dH_2O
    b. 2.5 mL 0.5M Tris HCl pH6.8
    c. 1.3 mL 30% Acrylamide/Bis Solution (Bio-Rad)
    d. 100 µL 10% SDS
    e. 100 µL 10% APS
    f. 10 µL TEMED

5. Pour stacking gel on top of polymerized running gel into gel cassette. Insert gel comb into stacking gel and let polymerize.
6. After polymerization, remove gel comb to generate wells and transfer gel cassettes to gel running dock as per manufacturer's instructions. Fill empty compartments with 1X Running Buffer.
7. Dilute desired protein samples 1:1 with 2X SDS running buffer and load into gel. Add ladder if desired.
8. Allow to run at 140V for 1:30 hours or until dye front is no longer visible.
9. Fill a basin with methanol and a second basin with 1X Trans-Blot Turbo Transfer Buffer.
10. Let PVDF membrane sit in methanol for 2 minutes, then transfer to transfer buffer basin with foam pads. Let sit for 2 minutes.
11. Assemble transfer according to manufacturer's protocol.
12. Transfer proteins from polyacrylamide gel to PVDF membrane using Turbo-Blot Transfer System.
13. Block 1 hour in 5% Milk
14. Incubate overnight with primary antibody in 5% milk
15. Wash 3 times with TBS-Tween
16. Incubate for 1 hour with secondary antibody in 5% milk
17. Wash 3 times with TBS-Tween
18. Develop the blot by preparing the ECL developing solution. Add ~1mL of ECL solution to the membrane and allow to incubate for 2 minutes on each side.
19. Image using the Bio-Rad ChemiDoc Gel Imaging System