Western Blot

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Abstract

To identify specific amino acid sequences of a protein, or tag, using fluorescentlytagged antibodies

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Protocol

Step 1.
Assemble gel polymerizing station according to manufacturer's protocol.

Step 2.

To create a running gel combine:

a. 2.4 mL dH2O

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

Step 3.
Pour into gel cassette and let polymerize.

Step 4.

To create a stacking gel combine:

a. 6.85 mL dH2O

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 TEMED2

Step 5.
Pour stacking gel on top of polymerized running gel into gel cassette. Insert gel combinto stacking gel and let polymerize.

Step 6.
After polymerization, remove gel comb to generate wells and transfer gel cassettes togel running dock as per manufacturer's instructions. Fill empty compartments with1X Running Buffer.

Step 7.
Dilute desired protein samples 1:1 with 2X SDS running buffer and load into gel. Addladder if desired.

Step 8.
Allow to run at 140V for 1:30 hours or until dye front is no longer visible.

Step 9.
Fill a basin with methanol and a second basin with 1X Trans-Blot Turbo TransferBuffer.

Step 10.
Let PVDF membrane sit in methanol for 2 minutes, then transfer to transfer bufferbasin with foam pads. Let sit for 2 minutes.

Step 11.
Assemble transfer according to manufacturer's protocol.

Step 12.
Transfer proteins from polyacrylamide gel to PVDF membrane using Turbo-BlotTransfer System.

Step 13.
Block 1 hour in 5% Milk

Step 14.
Incubate overnight with primary antibody in 5% milk

Step 15.
Wash 3 times with TBS-Tween

Step 16.
Incubate for 1 hour with secondary antibody in 5% milk

Step 17.
Wash 3 times with TBS-Tween

Step 18.
Develop the blot by preparing the ECL developing solution. Add 1mL of ECLsolution to the membrane and allow to incubate for 2 minutes on each side.

Step 19.
Image using the Bio-Rad ChemiDoc Gel Imaging System