Western Blot

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1 Works for me

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ABSTRACT

Biochemical technique to separate protein based on molecular weight. Assumed to have homogenized samples.

SDS-PAGE

1 Pull out comb gently and straight, avoid collapsing wells

2 Follow instructions provided with power pack to set up gels
- Short plate facing in on apparatus
- Press against gasket
- Press down and close arms simultaneously
- Put unit into bucket
- If not using 2 gels, use thick plast plate as the "dummy plate"

3 Fill inner chamber with 'fresh' 1x running buffer high enough to cover smaller plate and enter wells

4 Load protein ladder in right most lane
*Note: gels are reversed, final product will have ladder on leftmost lane

7 µl

5 Load samples with gel-laoding tips at consistent amount

Tips: avoid bubbles and draw/eject samples slowly

6 Pour "used" 1x running buffer to fill bucket

7 Place the lid on the tank with matched colour-coded leads. Run the machine until protein ladder sufficiently separated.

25 °C Room temperature

02:15:00 Constant 60 V

Prep Transfer Buffer (1L per container)

8 Make 1 L solution in "1x transfer buffer with MeOH" bottle

700 ml Distilled Water

200 ml Methanol

100 ml 10x Transfer Buffer

9 Put transfer buffer into -20^{o}C freezer #2 (bottom rack)

Transfer

10 Fill wide plastic tray with 1x transfer buffer

11 Soak filter papers (6 in total per cassette) in 1x transfer buffer in tray

12 Carefully remove gel from between glass plates. Use the green plastic wedge.

13 Using tweezers, obtain PVDF membrane and place in methanol on rocker. This activates the membrane.

14 Soak gels in transfer buffer for 5 minutes to equilibrate. Soak sponges as well.

15 Assemble immunoblot 'sandwich' in following order:

- Blot cassette with black (cathode) side down
- 2 sponges
- 3 sheets of soaked filter paper (roll out bubbles)
- Gel (with ladder on the right, dye front on the bottom)
- With tweezers, place PVDF membrane over gel
- 3 sheets of soaked filter paper (roll out bubbles)
- 2 sponges

Close the cassette. Leave the cassette in transfer buffer until all cassettes are assembled

16 Set up the power pack.

- Fill the bucket with the transfer buffer (should be about 3/4 full)
- Put in magnetic stir bar
- Put in ice pack into the bucket
- Ensure electrodes are in correct placement

Start reaction in the cold room. Small bubbles should be seen when the reaction starts

01:30:00 Constant 90V

Block

17 Block reagents and concentration will vary depending on antibodies.
Usually 10 mL of block solution per plate.

18 Place membrane onto plate with tweezers. Add used amido black. Place plate on the rocker for 1 minute.

19 Draw up excess amido black back into the bottle. Rinse the membrane with distilled water.

20 Cut the membrane with exacto-knife (if necessary). Take a picture.

21 Place membrane on rocker with TBS-T to keep the membrane moist.

22 Remove previous TBS-T. Add block solution and incubate on rocker
01:00:00 Incubate on rocker

Primary Antibody Incubation

23 Defrost primary antibody solution 5 mins before completion of block incubation

24 Remove block solution and add primary antibody solution.

25 Incubate on a rocker within the cold room **overnight**. Keep the bottle with the membrane for reference purposes.

Next Day

26 ---
14:00:00 Incubate with primary antibody (min)
18:00:00 Incubate with primary antibody (max)

Secondary Antibody Incubation

27 Remove primary antibody with 10mL pipette. Keep the solution and put it back into its respective tube.

28 Wash membrane:
Add 1x TBS-T to the membrane and incubate on the rocker
00:05:00 Wash/incubate on rocker

29 Perform wash step 3 times in total

30 Prepare secondary antibody solution. Diulent and % mass will vary depending on primary antibody used.
10 mL solution per plate.

Do not contaminate antibodies.

31 Add secondary antibody to the membrane
01:00:00 Incubate on rocker

32 Wash membrane:
Add 1x TBS-T to the membrane and incubate on the rocker

00:05:00 Wash/Incubate on rocker

33 Perform wash step 3 times in total

ECL Detection

34 Keep membranes in TBS-T but remove from the rocker while preparing the ECL solution.

35 Set-up the imager.
- Turn on the computer
- Autofocus with the Mac card

36 Clean the square plate with EtOH. Ensure no particular matter

37 Prepare ECL solution in a black tube. Mix using the pipette.
Use different pipette tips to prevent contamination.

750 µl Luminol

750 µl Clarity

38 Dap off excess TBS-T with KimWipe. Place membrane in square plate. Pipette out some of the ECL solution. In drop-wise fashion, just cover the membrane in the ECL solution.

39 Place container over the plate to avoid light

00:05:00 Incubate in solution

40 Using tweezers, put the membrane on the glass. Place the glass on the 3rd level in the machine in the middle of the tray.

41 Take preview image to see the ladder. Save the image.

42 Auto-expose and save the image

Membrane Storage

43 Soak membrane in 100% methanol and leave on a KimWipe to dry. Wrap the membrane in plastic wrap and label it.

25 °C Store at RT