

Western Blot

Materials:

Transfer buffer

Nitrocellulose cut to proper dimensions

Whatman paper-cut

A. Transfer proteins from gel to nitrocellulose

1. After dye has reached the top of the plug, turn off apparatus and remove plates. Ear plates are facing inside. Lane 1 is on the far left. Lay plates down such that lane 1 stays on the left. Remove bottom spacer. Separate plates using spacer: insert spacer at bottom right-hand corner and lift. Technique: only need a small portion of spacer to separate plates.

2. Whichever plate the gel sticks to, cut the bottom corner under lane 1, or the acid side on a 2nd dimension. Cut off wells from the stacking gel. Keep plug on as long as it doesn't make gel bigger than the nitrocellulose.

3. Label the bottom left-hand corner of nitrocellulose with the blot number. Pre-soak nitrocellulose in transfer buffer carefully and evenly. Let it soak for a minute. Only touch NC by the corners! Do not wrinkle! OK to soak Whatman in same tray.

4. Stack in the following order: Put transfer buffer between each layer. Remove bubble using 5ml glass pipet, especially between gel and NC.

untabbed side of cassette

thin sponge

2 Whatman

gel

NC

Whatman

thick sponge

tabbed side of cassette (close securely)

Lay flat and carry to cold room. Transfer at 0.500 Amps for 3.5-4 hours. Turn on stirrer. Tabbed side goes toward the back (SDS covered protein travels toward the positive electrode). Transfer buffer can be used for up to 6 gel transfers before it needs to be discarded.

B. Western Blot

1. After the transfer, block 1 hour in 100ml of 3% non-fat dry milk (NFDM). Block on shaker at room temp.

2. Rinse in PBST for 5min. Approx 20ml/blot.

3. Incubate for 2.5 hr with 1st antibody according to dilution given by DP. Record dilution, dates of usage, and add azide to 1st Ab. Save in glass frig. Incubate in roller or in tray.

4. Rinse with PBST for 5min 3x.

5. Incubate with 2nd Ab. 8l/40ml = 1:5000 dilution. Use rabbit or mouse HRP (in 4°C frig) depending on 1st Ab. Heavy chain or light chain.

6. Rinse 1x for 15min and 3x for 5min. Don't leave the blot in PBST for more than an hour before you do ECL or signal may look weaker.

7. Use ECL or Renaissance reagents to see proteins on film.