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Transfer of proteins from SDS-PAGE to 0.2 μm PVDF membrane (BioRad) using Idea Scientific's GENIE® Electrophoretic Transfer Apparatus

Read these following notes carefully before start.

1. Prepare the transfer buffer (Towbin buffer: 25 mM Tris-glycine, pH8.3/20% methanol): Tris-base 3.03 g; Glycine 14.4g, dilute to 800 mL ddH2O, mix well, then bring up to 1 L with 200 mL methanol. This buffer does not need pH adjust. (Note: under no circumstances will HCL be used to adjust pH since Cl- will erode the electrode).
2. Pre-soak minigel-sized hydrophobic PVDF membrane in 100% methanol for 30 seconds, then equilibrate the membrane in transfer buffer for at least 15 min before use.
3. Proteins size larger than 200 kDa or gels thicker than 1 mm may require extended transfer time. For proteins larger than 200 kDa, dilute the transfer buffer 1/2 , i.e. 12.5 mM Tris-Glycine, 10% methanol, then add 0.01-0.05% SDS to the diluted buffer, or eliminate methanol (since large proteins may be precipitated by methanol). The transfer time for proteins larger than 200 kDa is 1-2 hours @ 24 volts.
4. Protein size less than 20 kDa may pass through the membrane without binding. One can reduce the time and voltage of the transfer to 30 minutes @ 6 volts. The pore size of the PVDF may not exceed 0.2 μm.
5. For protein size in between (20<protein<200kDa), use 0.2 μm PVDF membrane, 25 mM Tris-Glycine/20% methanol. The time@voltage can be as follows: 60 min @ 12 V; 30 min @ 24 V; 30 min @ 6V followed by 90 min @ 24 V.

Assemble transfer sandwich:

1. Place the tray on a level surface.
2. Place one plastic bubble screen at the bottom of the tray.
3. Place the cathode (- lower electrode) at the bottom of the tray. The banana connector should be in the upper left hand side.
4. Place a plastic bubble screen, ribbed (rough, unsmooth) side down, on the cathode.
5. Place a SCOTCH-BRITE (a brand name from 3M) pad in the tray and fill the tray with the transfer buffer to the level even with the pad. Squeeze the pad to expel all bubbles.
6. Place a sheet of filter or chromatography paper (Whatman 3 MM), cut to the size of the pad, on top of the pad. Whatman 3 MM paper is thin, do not use "blotting papers" as they are thick and barriers to current.

7. Position the gel on the filter paper.
8. Place the pre-wetted transfer membrane on the gel. No bubble should be trapped underneath.
9. Rub the transfer membrane to expel all excess buffer from between the gel and the membrane. Failure to do so will result in fuzzy transfer. Do not have too much buffer in the tray to avoid the membrane floating off the gel.
10. Place a piece of filter paper over the membrane, be careful not to trap any bubbles.
11. Place Scotch-Brite pads over the filter paper, usually 2 new pads or up to five used, flattened pads. A second blot sandwich can be put between these pads.
12. Place a plastic bubble screen, ribbed side up, on the pad, and place the anode (+) on it. The banana connector should be in the right upper corner.
13. place the two-hole plastic anode cover over the anode.
14. The sandwich should be compressed about 2 mm to slide into the tray holder. If not, the gel may slide during the transfer. If this is the case, add more Scotch-Brite pads in between.
15. Keeping the tray level, slide the tray into the tray holder.
16. Slowly tip the GENIE to its vertical running position. Add buffer till it cover the transfer area.
17. Connect GENIE to the batter charger and begin transfer. Left-hand connection is (-) cathode, whereas right-hand connection (+) is anode.
18. Monitor the temperature during blotting.