

Protein Electrophoresis

Stop the gel. Disassemble the upper reservoir and remove the clamps from the gel.

Separate the glass plates from the gel by inserting the red plastic wedge device into the sandwich and twisting gently. Alternatively use this same device to slide one spacer out and then twist gently on the spacer until one plate is free. Set the glass plate, which sticks to the gel onto the bench so that the gel is facing up. Sometimes the gel will stick to both plates. Don't panic. Let the plates come back together and then try it again. Just be patient.

Separate any undesired portion of the gel from that which you wish to blot; i.e., if you do not need the entire gel; e.g., if your protein is toward the bottom of the gel, remove and discard the top portion (usually the stacking gel is peeled away from the resolving gel and discarded but if it does not come free, then follow the same procedure). To do this either cut the gel using one slicing action with a knife or alternatively, run a pizza slicer once across the gel (don't use a razor blade as this will shred the gel). Although the unwanted portion may be discarded in the normal waste since all acrylamide is polymerized, it is probably better to put it into the same container that the filter paper used to blot the H₂O-saturated isobutyl alcohol was put into.

Transfer the gel off of the bottom plate and into Towbin buffer in a container large enough to accommodate the gel. Sometimes the gel will stick to the bottom plate. Usually this is more of a problem with low concentration (<8% acrylamide) gels since the higher concentration acrylamide gels hold together quite well and can simply be picked up. However if the gel does stick and you cannot remove it from the plate, then simply hold the plate upside down over the Towbin buffer, touch the gel to the Towbin buffer and/or let the whole gel come into contact with the Towbin buffer. The gel should free itself of the plate.

Incubate the gel in a minimal volume of Towbin buffer on a shaking platform for 15 minutes.

Discard this Towbin buffer by hold the gel with one (gloved) hand and tipping the container over the sink.

Add another minimal volume of Towbin buffer and incubate for a second 15-minute interval. These incubations help to remove SDS from the gel, which sometime inhibits protein transfer out of the gel and onto the membrane.

Meanwhile prepare the GENIE ELECTROBLOTTER transfer apparatus and the transfer membrane. For proteins two membranes are typically used: nitrocellulose and PVDF, which is the same as the Amersham Hybond P that we have in the lab. (Not Hybond N, which is used for nucleic acid transfers.) PVDF has a higher protein binding capacity. However it always requires wetting first in methanol whether it straight out of the box or after the membrane has dried down after protein transfer.

Spread the roll of Hybond P out onto a clean area large enough to accommodate you. Have at hand a clean, sharp; i.e., new razorblade and a clean straight edge (wipe down a ruler with ethanol). Wear gloves. Wear gloves when handling both transfer membrane and the 3MM Chromatography papers. If oils from your hands contaminate them (especially the Hybond P), transfer will be inhibited. Transfer membranes are VERY EXPENSIVE! Open the roll such that one blue sheet is directly on the bench. The blue sheets provide protection to the membrane from dirt etc. You will probably need to weigh the membrane down so it does not roll back upon itself, just don't contaminate the unused portions. Measure out the size of membrane needed for the transfer. Simply mark the membrane with a pencil and then using the clean ruler as a straight edge and the new razorblade cut out only that portion of membrane you need. Gingerly label the membrane with a pencil. I generally label membranes in the lower left corner with pertinent info: experiment and date. Note that one side of the membrane will become the side that the protein binds to. It is therefore important that you are consistent when placing the membrane onto the gel to know which side was up against the gel and which side was not. This is critical when using the SURF-BLOT apparatus since one side of the membrane actually does not come into contact with any of the antibody solution. So, for example if you always label the membrane in the lower left hand corner with pencil and you always place the membrane onto the gel such that the pencil side is up, then the "pencil free" side of the membrane will face the gel and should be the side that gets exposed to the antibody.

Note that if the membrane is to be used with the SURF-BLOT apparatus it needs to be 10cm in length (same dimension as the gel lanes) but can be any width up to about 15cm.

Next place the cut out section of membrane into methanol to wet it. It only takes a few seconds to wet it. Remove the membrane from the methanol and place it into a small volume of Towbin buffer and incubate it along side the gel on a shaking platform until you are ready for the transfer.

(See also the GENIE ELECTROBLOTTER manual.)

Place the rectangular tray on a relatively level surface (your bench).

Place two plastic bubble screens in the bottom of the tray (optional).

Place the cathode plate electrode (this is the shiny plate with no writing on it) in the tray such that the banana connector is in the upper left hand corner.

Place a plastic bubble screen, ribbed side **DOWN**, on the cathode.

Place a SCOTCH -BRITE pad in the tray and fill the tray with Towbin buffer to a level even with the SCOTCH -BRITE pad. Squeeze the pad to expel all bubbles.

Place a sheet of Whatman 3MM Chromatography paper, cut to the size of the SCOTCH – BRITE pad (there is a box of precut Whatman 3MM in the cabinet) on the SCOTCH – BRITE pad. Be sure there are no bubbles trapped under the blotting paper.

Position the gel on the Whatman 3MM Chromatography paper.

Remove the transfer membrane from the Towbin buffer and place it onto the gel. Do this by holding the transfer membrane at opposite ends, and allowing the sagging center to touch the gel first. Slowly lower the ends. If done slightly below the level of transfer buffer, no bubbles should be trapped between the membrane and the gel. Air bubbles not expelled will result protein transfer out of the gel into the buffer and not onto the membrane.

Note that unlike DNA/Southern and RNA/northern blotting, no transfer of protein occurs until an electric field is applied; therefore if you need to lift up and reset the membrane it is okay to do so.

Rub the transfer membrane to remove all excess buffer from between the gel and the membrane. Failure to do this will result in fuzzy transfers. A glass rod can be used for this purpose. Do not have an excess of buffer in the tray, as this will tend to float the membrane off of the gel.

Place a sheet of Whatman 3MM Chromatography paper on top of the transfer membrane being careful to avoid trapping any air bubbles between the Whatman 3MM Chromatography paper and the transfer membrane.

Place SCOTCH –BRITE pads over top of the Whatman 3MM Chromatography paper. If the SCOTCH –BRITE pads are new then two may suffice. If they are older as many as five may be required since they flatten with age. Also note that additional blots and transfer membrane can be placed between the pads so that multiple gels can be blotted at once.

Place a plastic bubble screen, ribbed side **UP**, on the pad and place the anode (dull metal plate) such that the banana electrode is facing up in the upper right hand corner.

Position the plastic two-holed anode cover over the anode.

Compress the gel-blotting sandwich. Make sure that the plastic two-holed anode cover will fit into the rectangular tray.

Slide the sandwich into the tray holder.

Slowly tip the GENIE to the vertical position. Add buffer if the buffer level is below the blotting area.

Connect the GENIE to the battery charger. Left hand connection is (-); i.e., black. Right hand connection is (+); i.e., Red.

Turn battery charger to 12V and set the timer for 1 hour.

After transfer is complete, disassemble and rinse the GENIE.

The membrane may be allowed to dry on you lab bench or in a drawer until you are ready to use it. (Just remember that if it is PVDF that it requires wetting first in methanol prior to incubation in aqueous buffers.

Solutions, buffers and stuff:

GENIE ELECTROBLOTTER transfer apparatus

Whatman 3MM Chromatography paper

PVDF (Immobilon P) transfer membrane

Towbin buffer

<u>Stock</u>	(1L)	(6L)
(a) Tris Base	3.0g	18.17g
(b) Glycine-HCl	14.08g	84.48g
(c) dH ₂ O	800ml	4,800ml
(d) Methanol	200ml	1,200ml

DO NOT pH. DO NOT USE Commercial “Premixed” Buffers or any electroblotting buffers pHed using HCl with the GENIE ELECTROBLOTTER. The Cl⁻ will corrode the very expensive platinum electrode plates.