How to Use Bio-Rad Semi-Dry Transfer Apparatus for Western Blotting Analysis

(Quan Kang 8/28/03, Commented by TCH)

1. Run SDS-PAGE pre-cast gels (e.g., from Bio-Rad or ISC BioEXpress) as usual.
2. Prepare transfer membrane: cut the PVDF membrane to the dimension of the gel. Wet the membrane by soak it into 100% methanol 10~15 sec, then wash it with distilled water 3 times, keep the membrane in Transfer Buffer (**Note: You must first wet the membrane in 100% methanol and never let it dry afterward**).
3. Remove safety cover and stainless steel cathode. Assemble the transfer sandwich in the following order (from the bottom platinum anode up): one piece of pre-wet extra thick filter paper (or3~6 general thick filter papers) >>> Pre-wet PVDF membrane >>> SDS-PAGE gel (**Optional: the gel can be pre-equilibrated in the Transfer Buffer for 5-20min**) >>> one piece of pre-wet extra thick filter paper (or3~6 general thick filter papers). **Note: Roll out air bubbles between layers with a 5ml pipette after assembly the sandwich.**
4. Secure top stainless steel cathode and safety cover. Start the transfer using the following suggested conditions: **Mini gel**, 10-15V, 250mA for 20min (high MW proteins, run 25~30min); **Large gel**, 25V, 250mA for 30min.
5. Turn off the power supply, unplug electrodes, and remove blot. Complete Western blotting by following the conventional Western Blotting protocol.

Tips:

1) The PVDF membrane and filter papers must be 100% equilibrated.
2) The filter papers should be cut to the exact size of the gel (this forces the current to flow only through the gel and not through overlapping filter paper).
3) Any bubbles between the layers will block current flow and prevent protein transfer.
4) Once assembled, do not move the top stainless cathode, any shifting of the transfer stack after assembly will distort the transfer pattern.

5) Multiple gels can be transferred, simply put a sheet of porous cellophane or dialysis membrane equilibrated with Transfer Buffer between each transfer sandwich.

Transfer Buffer (for PVDF membrane)

800 ml Methanol (final concentration = 20%)
12.12g Tris Base
57.63g Glycine
Add ddH2O to 4 Liters
(Note: pH should be 8.3~8.6, but NEVER add acid or base to adjust it).