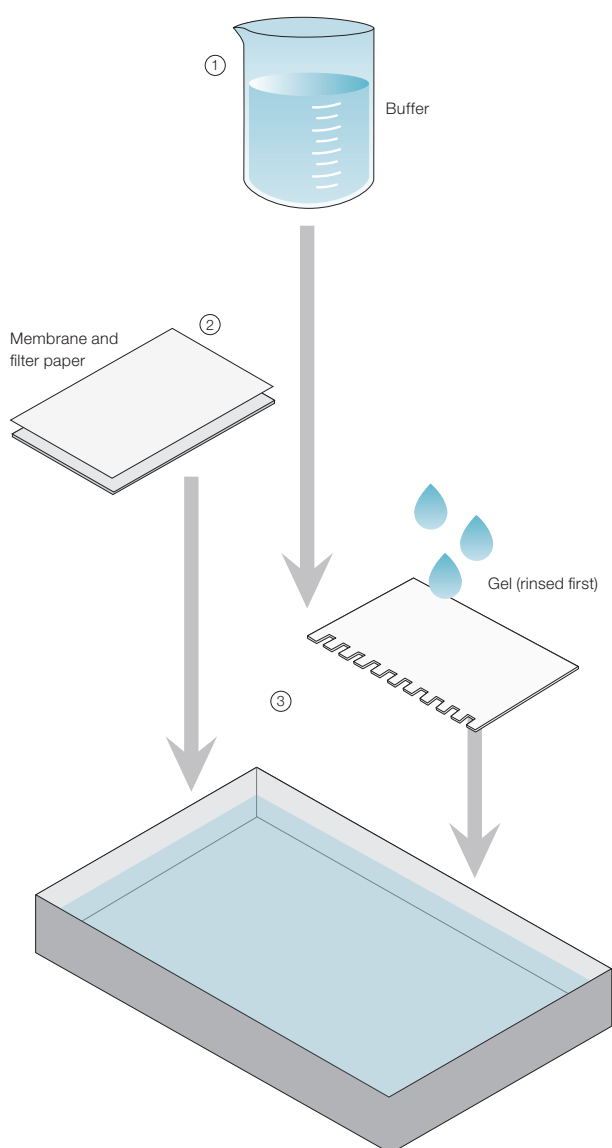


Reagent and Materials Preparation



- 1** Prepare transfer buffer. Refer to Table 2.2 or the instruction manual for the transfer cell you are using for the buffer requirements of each transfer unit. Add 200 ml of buffer per gel for equilibration of gels and transfer materials.
- 2** Determine the proper membrane for the specific experiment. Select a precut membrane and filter papers or cut the membrane and filter paper to match the size of the gel.
- 3** Equilibrate (if necessary). Wet and equilibrate membranes in transfer buffer for at least 5 min. For PVDF membranes, wet in 100% methanol for ~1 min prior to equilibration in transfer buffer. Rinse gels in diH₂O and equilibrate in transfer buffer for 15 min.

TIPS

Gel equilibration removes contaminating electrophoresis buffer salts. If not removed, these salts increase the conductivity of the transfer buffer and the amount of heat generated during transfer.

Equilibration also allows the gel to adjust to its final size prior to electrophoretic transfer. Gels shrink or swell to various degrees in the transfer buffer depending on the acrylamide percentage and the buffer composition.

Equilibration is not necessary (i) when the same buffer is used for both electrophoresis and transfer (for example, native gel transfers), or (ii) when using rapid semi-dry transfer systems such as the Trans-Blot® Turbo™ system (consult the user manual for the system you are using).

This is an excerpt from Bio-Rad's comprehensive Protein Blotting Guide (Bulletin 2895).



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