

## Transfer of Protamines from Acid-Urea Gels to Immobilon-P (Western Blot)

[Protocol Developed by Michele Corzett, Lawrence Livermore National Laboratory]

Note: Wear powderless gloves when handling the gels and transfer membranes

1. Place enough transfer solution (0.0009N acetic acid) in a clean photographic tray to cover the bottom of the tray.
2. Wet two pieces of Immobilon-P membrane (Millipore) for each gel to be transferred (precut to size) with methanol and place in the transfer solution.
3. Soak two precut pieces of filter paper and three scotchbrite pads (by squeezing) in transfer solution for each gel to be transferred.
4. After unloading the gel from the cassette, mark lane 1 by punching holes in the gel above the lane, cut off the thick area on the bottom of the gel, and soak the gel in transfer solution for 10 min.
5. Assemble the following stack of pads, filter paper, membrane and gel in the following order, keeping everything wet and without introducing air bubbles:
  - Scotchbrite
  - Filter paper
  - Gel (hole on left side)
  - Immobilon-P membrane (with notch cut out on left side)
  - Second piece of Immobilon-P membrane
  - Filter paper
  - Scotchbrite

If transferring two gels at the same time, continue the stack:

- Filter paper
  - Gel #2 (two holes on left side)
  - Immobilon-P membrane (with two notches cut out on left side)
  - Second piece of Immobilon-P membrane
  - Filter paper
  - Scotchbrite
1. Insert the gel/membrane/filterpaper/scotchbrite 'sandwich' in the transfer holder carefully to prevent the layers from slipping. If needed, add more scotchbrites on the outside of the 'sandwich' to get a tight fit in the holder and apply the top to the transfer holder.
  2. Fill the gel box to half full with transfer solution and place the transfer holder with the gel 'sandwich' in the gel box. Attach the electrodes and confirm they are in the right orientation to move the protamines toward the Immobilon-P membrane.
  3. Fill the gel box with transfer solution until the gel is totally covered.
  4. Turn on power and perform transfer at 50V for 15 min.

Disassemble the 'sandwich', remove the membrane(s) with the notches, place on white absorbent paper towels and let dry.