Immunostaining of Western Blots using Anti-protamine Antibodies

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

Note: Wear powderless gloves when handling the transfer membranes

1. Prepare a blocking solution containing the following (this is enough solution for 4 blots): 5 gm bovine serum albumin (Fraction V, RIA grade), 100 ml Solution A (0.M Tris pH 7.4, 0.1% TWEEN 20, 0.9% sodium chloride) and 200 μl calf thymus DNA (10 mg/ml) in water.
2. Turn on and set hybridization oven at 37 °C.
3. Wet the Immobilon-P membrane gel transfer with methanol and rinse the blot with water.
4. Place the membrane in a 50 ml disposable plastic screw cap tube, add 25 ml of blocking solution, cap the tube, and place it in the hybridization oven at 37 °C for one hour with rotation
5. Remove the tubes from the oven, cool to room temperature, and discard the blocking solution.
6. Wash each membrane while in the tube with 40 ml of Solution A; repeat two additional times and drain thoroughly.
7. Add 10μl of the primary antibody (Hup 1M, Hup 1N or Hup 2B) to 10 ml Solution A and transfer this to the tube containing the membrane. Return the tube to the hybridization oven and incubate at ambient temperature for 2 hr with rotation.
8. Wash the membrane (while in the tube) with 40 ml Solution A, repeat two additional times, and then add 10 ml fresh Solution A to the tube and membrane.
9. Add 35 μl biotinylated anti-mouse IgG secondary antibody to the tube and return to the hybridization oven for 30 min at room temperature with rotation.
10. Prepare the substrate immediately and let sit for 30 min. The substrate contains 10 ml Solution A, 2 drops Reagent A and 2 drops Reagent B from an ABC-AP kit (Vector Laboratories).
11. Wash the membrane (while in the tube) with 40 ml Solution A, repeat two additional times, and then add the 10 ml of the ABC-AP solution to the tube and membrane. Return to the oven (at RT) for 30 min with rotation.
12. Wash the membrane (while in the tube) with 40 ml Solution B (0.1M Tris pH 7.4, 0.9% sodium chloride) and repeat two additional times.
13. Prepare 10 ml of Vector Red substrate (Vector Laboratories) containing 10 ml 100mM Tris pH8, 4 drops Reagent 1, 4 drops Reagent 2 and 4 drops Reagent 3 as described in the instructions provided with the reagents and add to the tube and membrane. Invert the tube to flow substrate over the membrane until the color begins to develop.
14. Transfer the membrane to a petri dish and watch until color is fully developed (but before the background becomes too dark). Pour off the substrate and wash the membrane in water for 5 min. Photograph.

To probe with a second primary antibody, re-wet the membrane with methanol, rinse with water and add the primary antibody (step 7). There is no need to reblock. Continue as with first antibody, but use the Vector Blue substrate to visualize the second antibody.