BioPharmaceutical Technology Center
I N S T I T U T E

Immunology Field Trip

Protocol

I. Block Membrane

Note: Always wear gloves and use forceps when handling nitrocellulose membranes.

1. Label the weigh boat containing the membrane with your initials.
2. Add **5 ml Blocking Solution (PBS + 3% BSA, labelled 'BLOCK')** to the membrane and incubate at room temperature with gentle agitation for at least 10 minutes.

II. Purification of IgY Antibodies from Egg Yolks

1. Students should label **two** 1.5ml tubes with their initials.
2. Place a folded paper towel into a weigh boat. This is for the egg yolk.
3. Crack a room temperature egg over the 1L glass beaker (or container). Save the yolk and separate the egg white into the beaker. Place the yolk on the paper towel.
4. Using a transfer pipet, puncture the yolk and take up **2 ml of yolk** and add it to the tube containing 8 ml of **Precipitation Buffer A (larger tube, labelled 'PPTA')**. (10 ml total volume)
5. Cap the tube and **gently** mix the yolk with the buffer by inverting the tube for **2 minutes**.
6. Place the gauze pad over the top of the 100 ml beaker (or small container) to form a filter. Filter the mixture by carefully pouring the contents of the tube through the gauze and into the beaker.
7. After filtering, remove the gauze to the egg-waste container.
8. Using a P1000 Pipetman and blue tip, add **1 ml of the filtered yolk mixture** into **one** of your labeled 1.5 ml tubes.
9. Centrifuge the tube in a balanced, tabletop microfuge for 10 minutes.
10. Carefully remove the tubes from the centrifuge so that the pellet is not disturbed. Pipet **500 µl of the supernatant** into your other, clean, labeled 1.5ml tube.
11. With a P1000 and a clean pipet tip, add **100 µl of Precipitation Buffer B (labelled 'PPTB')** to the 500 µl of supernatant.
12. Cap the tube and **gently** mix by inverting and flicking the tube several times.
13. Centrifuge for 5 or 10 minutes.

BioPharmaceutical Technology Center
I N S T I T U T E

II. Purification of IgY Antibodies from Egg Yolks (continued)

14. Without touching the pellet, use a P1000 to pipette off most of the supernatant. Discard the tip and the liquid in the waste beaker.

15. Use a P200 and a yellow tip to carefully remove any remaining liquid from the tube.

16. **Add 1 ml of 1X PBS** (from the 50 ml tube) to resuspend the pellet by performing the following steps in order:

1) Add 500 µl PBS to the antibody-containing pellet using a p1000 and a blue tip.
2) Use the tip of the pipette to scrape at the pellet (or the place where the pellet should be) to release it from the side of the tube. Use the pipette tip to mix by drawing and dispensing several times.
3) Add another 500 µl PBS to the tube and cap it.
4) Vortex the tube for ten to fifteen seconds.

17. Retrieve your membrane from the rocking platform and proceed to Part III below.

III. Using Purified IgY to Detect Proteins

1. Pour the Blocking Solution back into its tube; leave the membrane in the weigh boat.

2. Pipet **1 ml IgY antibodies** (P1000) onto membrane and incubate on a rocker for 15 minutes.

3. Pour off the IgY solution into the waste beaker.

4. Wash the membrane by pouring about **10 ml of PBS** onto it; gently shake for 2 minutes.

5. Pour the PBS into the waste beaker and repeat the wash with another **10 ml PBS**.

6. Pour the PBS into the waste beaker and add **4 ml HRP-conjugated, rabbit anti-IgY (labelled 2°ab).**

7. Incubate the membrane and this secondary antibody on a rocker for 15mins.

8. Pour the secondary antibody (2°ab) back into its tube.

9. Wash the membrane by pouring about **10 ml of PBS** onto it; gently shake for 2mins.

10. Pour the PBS into the waste beaker and repeat the wash 2 more times with **10 ml PBS.**

11. Pour the PBS into the waste beaker and pipet **750 µl of the TMB (HRP Substrate)** onto the membrane.

12. Allow the color to develop, and then pour off the substrate solution.

13. Quickly rinse the membrane twice with ddH_2O to completely remove the substrate.

14. Dry the membrane; wrap the blot in plastic wrap to preserve it. The color bleaches if the filter is not stored in the dark. You can take a photo of your membrane result. In that case, discard the membrane into waste container.