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.
3. Place weigh boat on a rocking platform for at least 10 min.

II. Purification of IgY Antibodies from Egg Yolks

1. Place a folded paper towel into a weigh boat or large plastic lid.
2. Crack a room temperature egg. Separate the yolk from the white; placing the yolk on the paper towel to save and the white into the orange biohazard bag.
3. Use a transfer pipet to puncture and suck up the yolk.
4. Add **yolk** to the tube containing 8ml **precipitation buffer A (PPTA)** until the total volume in the tube is 10ml.
5. Cap the tube and *gently* mix by inverting for **2 min**.
6. Place a gauze pad over the top of the small glass jar. Press down in the center of the gauze to make a depression to hold the liquid.
7. Filter the mixture by carefully pouring the contents of the tube through the gauze and into the beaker.
8. When filtering is complete, place the gauze in the orange biohazard bag.
9. Label a 1.5ml microcentrifuge tube with your initials and **"A"**
10. Using a P1000 micropipette and a blue tip, measure **1000µl** (1ml) of the **filtered yolk mixture** and place into the microcentrifuge tube.
11. Centrifuge the tube in a balanced tabletop microcentrifuge for 10 minutes.
12. Carefully remove the tubes from the centrifuge so that the pellet is not disturbed.
13. Label a 1.5ml microcentrifuge tube with your initials and **"B"**
14. Use a P1000 to pipet **500µl of the supernatant** into the B tube you just labeled.
15. Use a P1000 to add **100µl of precipitation buffer B (PPTB)** to tube B.
16. Cap the tube and *gently* mix by inverting and flicking the tube several times.
17. Centrifuge for 5-10 minutes.
18. Avoid touching or disturbing the pellet. Use a P1000 to remove most of the supernatant. Discard the tip and the liquid in the waste beaker.
19. Use a P200 set to ~100µl and a yellow tip to carefully remove any remaining liquid from the tube, continuing to avoid the pellet. Discard the tip and liquid in the waste container.
20. Resuspend the antibody-containing pellet by adding **500µl PBS** to tube B. Use a P1000 and a blue tip.

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

21. Use the tip of the micropipette to scrape at the pellet to release it from the side of the tube. Pipette the liquid up and down to mix.
22. Add another 500µl PBS to the tube and cap it.
23. Vortex the tube for 10-15 sec.

III. Using Purified IgY to Detect Proteins

1. Retrieve your membrane from the rocking platform.
2. Pour the blocking solution in the weigh boat back into its tube. Leave the membrane in the weigh boat.
3. Pipet 1000µl isolated IgY antibodies (tube B) onto the membrane. Incubate on the rocking platform for 15 minutes.
4. Pour off the IgY solution in the weigh boat into the waste beaker.
5. Wash the membrane by pouring about **10ml of PBS** onto it. Incubate on a rocker for 2 minutes.
6. Pour the PBS wash in the weigh boat into the waste beaker. Repeat the wash with another **10ml PBS.**
7. Pour the PBS wash into the waste beaker.
8. Add **4ml HRP-conjugated rabbit anti-IgY (secondary)**. Incubate on a rocker for 15 min.
9. Pour the secondary antibody back into its tube.
10. Wash the membrane by pouring about **10ml of PBS** onto it. Gently rock for 2 min.
11. Pour the PBS wash in the weigh boat into the waste beaker. Repeat the wash with another **10ml PBS** and gently rock for 2 min.
12. Remove the PBS wash into the waste beaker then repeat with one additional wash with **10ml PBS**.
13. Pour the final PBS wash into the waste beaker and pipet **750µl TMB** (HRP substrate) onto the membrane.
14. Allow the color to develop. Pour off the substrate solution once color is clearly visible.
15. Quickly rinse the membrane twice with **nanopure water** to completely remove the substrate.
16. Photograph the membrane if you wish to document your results.