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 **5ml 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 pipette to puncture and suck up the yolk.
4. Add **yolk** to the tube of **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 or beaker. Press down in the center of the gauze to make a depression to hold the liquid.
7. Filter the mixture by 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 or your name.
10. Use a transfer pipette and the markings on the side of the tube to add 1ml (1.0) of the **filtered yolk mixture** into the microcentrifuge tube.
11. Centrifuge the tube in a balanced tabletop microcentrifuge for 5-10 minutes.
12. Carefully remove the tubes from the centrifuge so that the pellet is not disturbed.
13. Label another 1.5ml microcentrifuge tube with your initials or your name.
14. Use a P200 to add **100µl of precipitation buffer B (PPTB)** to the tube with your supernatant.
15. Use a P1000 to add **500µl of the supernatant** into the tube you just labeled.
16. Cap the tube and *gently* mix by inverting and flicking the tube several times.
17. Centrifuge for 5-10 minutes. Make sure the hinge of your tube faces outwards.
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 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 1x PBS Wash** to your tube.
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.

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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. Pipette 1000µl isolated IgY antibodies 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.

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