SIMPLIFIED PURIFICATION OF RABBIT IGG

Created/updated by: Kyle Novakowski Date: September 6th 2012
Bowdish Lab, McMaster University
Hamilton, ON, Canada
www.bowdish.ca

BACKGROUND

- This protocol is used to purify serum that has been collected from an immunized rabbit for the purpose of creating an antibody. This protocol uses protein-A sepharose beads, which originate from *Staphylococcus aureus*. Protein A serves to protect the bacteria by binding IgG antibodies in the opposite direction and therefore can avoid opsonization and phagocytosis. Antibody purification exploits the use of protein-A by coupling it to sepharose beads where a crude mixture such as serum can be run through. The IgG antibodies found in the rabbit serum will thus bind to the protein-A and will allow for isolation and purification of the antibody to be used for research purposes.

NOTES

- This is a partial protocol assuming the beads have already been swelled and properly packed into a column.
- A second pair of hands is very handy during the elution step.
- It is best to make all solutions fresh (or no older than a week) to ensure proper binding and elution of IgG.
- Never let the column run dry, this will wreck the beads. Always have a minimum of 1-2mL of liquid above the bed.
- Whenever loading anything into the column, do so gently so as not to disturb the bed.

EQUIPMENT

- 300mL Sodium Phosphate, 20mM pH = 7.0 (best to make extra in case) (see recipe section)
- 50mL Glycine, 100mM, pH = 2.7 (see recipe section)
- 50mL TRIS, 1M, pH= 9.0 (see recipe section)
- Pre-packed protein-A sepharose beads in a column stored with "Storage buffer"
- 70% Ethanol
- Serum (Be sure to check the animal the serum was taken from is compatible with the beads)
- Eppendorf 1.5mL tubes
- Clean 125mL beakers
- 1.5L PBS
- 2L beaker
- Stir bar
- Styrofoam (if using a dialysis cassette)
- Dialysis tubing (if not using a cassette)
- 21 gauge syringes
- This procedure requires 2 days. One for collection & purficication and overnight/day two for dialyzation

PROTOCOL

1. If serum is frozen, thaw.
2. Unscrew bottom stopper and quickly place tube into a waste beaker. Run 100mL pH 7.0 Sodium Phosphate through column to elute the storage buffer.
3. Stop the flow when 1-2mL buffer is left above the bed. Remove the waste beaker and place a new clean beaker under the column.

4. Load the serum into the column until it is full. Then unscrew the bottom stopper and drain the serum into the new beaker. Run the serum 2 more times (using an additional clean beaker in between) for a total of three times.
5. The antibodies should be bound to the column, however in case something has gone wrong keep the flow through. It may still have lots of antibodies in it.
6. Switching back to the waste beaker, unscrew the bottom cap and run 100mL pH 7.0 Sodium Phosphate through the column. Stop the flow when 1-2mL buffer is left above the bed. Remove the waste beaker and place a new clean beaker under the column.
7. Determine the ratio of 1M TRIS pH = 9.0 to 100mM Glycine pH = 2.7 in order to get a neutral 1mL solution. This is typically approximately 50-55uL TRIS and 945-950uL Glycine. This is so when the antibodies are eluted using acidic Glycine, the fractions can be placed directly into pre-aliquotted TRIS in order to neutralize the solution rapidly. Use pH paper to determine the correct ratio.
8. Label 25 eppendorf tubes #1-25 and aliquot the determined amount of 1M TRIS pH = 9.0 into each.
9. With a second person in charge of vortexing and closing the tubes ready, load 20mL 100mM Glycine pH= 2.7 and unscrew the stopper. Fill the first tube to the 1mL mark and quickly switch to the second tube. At the same time, pass the first tube to the helper who will quickly close the tube and vortex it 3-4 times. Repeat until 1-2mL Glycine is left in the column and screw in the stopper.
10. Test the protein fractions on the microdrop, using a neutral TRIS/Glycine mix as the blank. Keep the best 4-8 protein concentrations, with a minimum of around 0.3mg/mL. Record the concentrations and pool the fractions.
11. Load the solution into a dialysis membrane cassette using a 21 gauge syringe. Be extra careful not to puncture the membrane. Dialysis tubing can be used alternatively. If using a cassette, attach it to a piece of Styrofoam.
12. Fill a 2L beaker with around 1.5L PBS and add a stir bar. Carefully place the casstte/Styrofoam piece into the solution. Let this gently stir at 4°C overnight.
13. The next morning, change the PBS and let stir for an additional 2 hours.
14. Remove the solution from the cassette using a 21 gauge syringe and aliquot the antibody. If storing at 4°C, add 0.01% Sodium Azide to prevent contamination. This is not required for aliquots stored at -20°C.

RECIPES

- 100mM Glycine pH = 2.7 → Dissolve 0.3754g Glycine in 50mL distilled water.
- 1M TRIS pH = 9.0 → Dissolve 12.114g TRIS in 50mL distilled water.
- 20mM Sodium Phosphate pH= 7.0 → Dissolve 0.071g Disodium Orthophosphate and 0.069g Monosodium Orthophosphate into 100mL of distilled water. Size up accordingly. Best to make 1L.

LINKS AND REFERENCES

- www.bowdish.ca/lab