

Affinity Purification of Polyclonal Antisera

Protocol

- 1) Run denaturing polyacrylamide gel with 10µg to 100µg of the purified protein used to generate antisera. Typical load is 1-10µg per well or 10 to 100µg total in one large well.
- 2) Transfer protein to PVDF membrane using standard procedure for immunoblotting.
- 3) Stain the membrane with Ponceau S. Rinse with ddH₂O to destain. Using a razor blade or X-acto knife cut out the region of the membrane containing the protein trying to get as thin a strip as possible.
- 4) Block the membrane with 5% dry milk in PBS for 20 min at RT.
- 5) Wash membrane 2 X 2 minutes in PBS. Cut membrane in two. If only affinity purifying a single ml of sera it is possible to use only half of the membrane and save half, tightly wrapped in plastic, at -20°C. Re-wet frozen membrane in MeOH and rinse in PBS before using.
- 6) Incubate strip of membrane containing protein with 300µl of antisera for 1 hour at room temperature. It is helpful to cut the strip of membrane into smaller pieces and put them into a microfuge tube for incubation purposes.
- 7) After incubation, pull off the depleted antisera and set aside. Antisera may retain a relatively high titer of antibodies and can be subjected to further affinity purification should serum be in short supply.
- 8) Wash the membrane 2 X 15 minutes in 5ml of PBS.
- 9) Meanwhile, aliquot 100µl of NaPO₄ pH 8.0 into 3 microfuge tubes.
- 10) To strip membrane add 300µl of 5mM glycine 150mM NaCl pH 2.4.
- 11) Incubate 30 seconds at room temp.
- 12) Pull off supernatant and put into microfuge tube with NaPO₄ solution to neutralize.
- 13) Repeat steps 10-12 two more times.
- 14) Combine all three tubes to equilibrate the antibody levels and then divide into fresh aliquots. Generally, dialysis of affinity purified antisera does not appear to be necessary. Store the affinity purified antisera at 4°C as freezing sometimes damages the antibody. Sodium azide can be added to 1mM.

Yields vary so it is important to titer the antibody after each affinity purification.

Solutions

PBS

Ponceau S

(10X solution: 2% Ponceau S in 30% trichloroacetic acid, 30% sulfosalicylic acid)

5% dry milk in PBS

Stripping solution

(5mM glycine, 150mM NaCl pH2.4)

1M NaPO₄

Supplies

polyacrylamide gel

PVDF membrane