STRIP AFFINITY PURIFICATION OF ANTIBODIES

1. Pour an acrylamide gel with single well at each end and all the middle wells taped together.

2. Load molecular weight markers into the 2 single wells and fill the middle well with target protein.

3. Run and blot gel onto 0.2 μ nitrocellulose membrane.

4. Block membrane with appropriate blocking agent and incubate with 1° antibody stock overnight at 4 °C.

5. Next day, remove antibody and wash 3 x with wash solution as for normal western blot.

6. Cut strips from both ends of the membrane including the markers and a small amount of the large well content. Remember to mark strips so you can orientate them correctly.

7. Incubate strips with 2° antibody then wash and develop western as normal.

8. Once you can visualise the region of the membrane containing the antibody, place strips next to rest of membrane and cut out this region.

9. Place this strip onto some parafilm that has been pressed onto the bottom of a petri-dish. Surround the edge of the dish with wet paper towels.

10. Overlay the membrane with a small volume of **elution buffer** (50-200μl) and allow to shake very gently at room temp for 20'.

11. Remove the **elution buffer** and neutralise with 0.1 volumes of 1M Tris base.

12. Add an equal volume of **PBS** and 0.02% sodium azide. Store at -80°C for long-term storage

Elution buffer (50 ml)

0.2M glycine pH2.8	10 ml 1M
1mM EGTA	100 µl 0.5 M

10 x PBS (1L)

100 mM sodium phosphate pH 7.0	100 ml 1 M
1.5 M NaCl	300 ml 5 M