

Affinity Purification of Antibodies

(as performed by Jeff Cooper in the Price Lab)

This protocol uses 1 mg of protein, however it can be scaled up or down.

Check Sterogene information for compatible buffers (NO PRIMARY AMINES!)

Pipet 2 ml Sterogene Actigel ALD Resin (50% slurry) into 15 ml centrifuge tube

After a slow (1000 rpm in J6), short (2 min.) centrifugation, remove the liquid

Resuspend resin with 3 ml of the same buffer the protein is in

Spin again, remove liquid and resuspend resin with 2 ml of the same buffer

Transfer to 10 ml BioRad disposable column

Add protein to beads. Add ALD Coupling Solution to make the sample 100 mM ALD CS. Rotate column for 1-2 hrs at room temp.

During this incubation, heat 5 ml antiserum at 55°C for 30 minutes.

Filter the antiserum through a 0.45 µm syringe-driven filter

Allow the unbound protein to flow through the column (save in a 15 ml tube). Check OD280 (should be <0.1).

Wash resin with 5 ml PBS

Pass the heat treated antiserum over column 6 times.

Wash resin with 20 ml PBS. Check OD280. (should be <0.1)

Mix 200 µl 1 M Tris pH 10.4 with 550 µl 1 M KCl.

Pipet 75 µl into each of eight microfuge tubes used to collect elutions to quickly neutralize eluted material (check to make sure that 75 µl is enough to neutralize 0.5 ml of the elution buffer)

Elute with 100 mM glycine pH 2.5 in 0.5 ml fractions (store at -80°C in small aliquots; good for years this way).

Analyze purified IgGs by silver stained SDS PAGE

Test affinity purified antibody compared to antiserum by western blot with pure protein and crude extract on the blot.

