AlphaGenix
6740 Nancy Ridge Drive • San Diego, CA 92121
Tel: 858-458-6817 • Fax: 858-458-6819

# Affinity Purification Protocol

This purification gel (thiol coupling/agarose gel or amino link gel) in a phosphate buffer contains 0.05% sodium azide (storage buffer) and should be refrigerated upon receipt. The purification gel has a shelf life of at least 6 months if stored at 4°C. Do not freeze. (Freezing will destroy the molecular bonds formed between the peptide and the gel).

**Buffers Needed**

| | |
|---|---|
| Salt Buffer: | 50 Mm $NaH_2PO_4$, 0.5 M NaCl **pH 6.5** |
| Phosphate Buffer: | 50 Mm $NaH_2PO_4$/Na2HPO4 (73.5% mono, 26.5% di) pH **6.5** |
| Glycine Buffer: | 100 Mm Glycine-HCl **pH 2.5** |
| Dialysis Buffer: | Dulbecco's Phosphate Buffered Saline (DPBS) w/o Ca/Mg **pH 7.4** |
| Storage Buffer: | Phosphate buffer plus 0.05% sodium azide |
| Tris Buffer: | 1 M Tris **pH 9.5** |

**Equipment Needed**

| | |
|---|---|
| Biorad Econo-Column | (BioRad cat #737-1011) diameter: 1.0 cm, length: 10.0 cm, cross-sectional area: 0.79 $cm^2$, volume: 8 ml |
| Extra End Caps | (BioRad cat#731-1660) this item optional |
| Tygon tubing | (BioRad cat#731-8215) |
| Acetal tubing clamp | (VWR cat#182270-0000) |
| Reducer | (Daigger cat#AKP0206CA12P) 1/16 x 1/8 1M/CS |
| End-Over-End Rocker | (VWR cat#62404-006), or orbital shaker (for mixing columns) |
| Dialysis tubing | (VWR cat#25223-800) Spectra/Por Membrane MWCO: 10,000 |
| Dialysis tubing clips | |

## Affinity Purification of Antibody

### Antibody Binding

1. Wash column with 10 column volumes of phosphate buffer (25 ml of buffer per 2.5 ml gel).

2. Mix up to 50 ml of serum with the gel in 50 ml conical.

3. Incubate at 4°C overnight on a end-over-end rocker (or for 2 hours at room temperature).

## Elution of Antibody

4. Pour the gel and serum back into the column. Allow gel to settle for approximately 30 minutes.

5. When gel has settled, collect the flow-through (FT) and label. Store FT at 4°C for later use.

6. Wash column with 25 column volumes of salt buffer to remove non-specific antibody (60ml of buffer per 2.5 ml gel.).

7. Elute antibody with glycine buffer. Collect 10mls in a 50-ml conical containing 0.733-ml Tris Buffer pH 9.5. This is enough base to neutralize the sample.

8. Dialyze the eluted antibody in DPBS, at 4°C, with one buffer change.
   Change should be at least 4 hours after antibody is put on dialysis and before the end of the day. If time does not permit this, change PBS first thing the following morning and wait at least 4 more hours before removing antibody from dialysis.

9. Place dialyzed antibody in a 15 ml conical and keep on ice for remainder of protocol
   (if volume exceeds 12 mls, use a 50 ml conical)

10. Check for any precipitate of the antibody. If precipiate is present, spin purified antibody in temperature controlled centrifuge @ 2000rpm for ~5 minutes. Pour off the antibody into a fresh conical, tossing out the conical with the pellet.

11. Check and record the OD @ 280 nm.

12. Quantitate Antibody: $A_{280}$ method

    Since a 1-ml sample of affinity purified antibody was analyzed spectrophotometrically at 280 nm through a 1-cm path, the following calculation should be used:

$$\frac{\text{O.D. x dilution factor}}{1.43} = \text{concentration of purified antibody}$$

$$\text{Concentration x volume} = \text{total milligram yield}$$

13. Aliquot the dialyzed antibody as needed and freeze.

14. Regenerate purification column by washing the column with 5-10 mls of storage buffer. Leave at least an equal volume of storage buffer in the column, so that column remains hydrated throughout storage. Store upright at 4°C.