AFFINITY PURIFICATION OF ANTIBIODIES USING HIS-TAGGED PROTEIN

THINGS YOU MUST KNOW FIRST:

- solubility of protein in TBS or Guanidine/Urea
- HIS-tagged protein does not elute from column in TBS or 4M $MgCl_2$
- rough idea of capacity of Ni-agarose for antigen (normally 5-10mg per 1 ml resin). Want to aim to load resin to 80% capacity.

NOTE: It is possible to start this protocol with crude bacterial extract and essentially purify the HIS-tagged protein on the same column as you affinity purify the antibody. However, you get a better of idea of binding efficiency if you first purify the HIS-tagged protein, quantify the amount eluted and reload a new column as below.

1. Mix 8 mg purified HIS-tagged protein (that has been eluted from a Ni column in **Buffer E**) with **Buffer B**, to make a final volume of 8 ml. Adjust pH to 8.0 if necessary.

2. Add 2 ml 50% Ni-agarose slurry and shake at room temperature for 1h.

3. Decant protein/bead slurry into a column. Allow column to drain by gravity flow until just above top of beads.

4. Wash column with 10 ml (i.e. 10 column volumes) **equilibration buffer**.

5. Load 2 ml crude serum onto column. Collect flow through & reload 3 x. (Check activity of final FT on western – if it still reacts against the antigen, you have overloaded the column with serum).

6. Wash column with 5 ml (5 column volumes) **equilibration buffer** then 5 ml of: 2 M NaCl, 50 mM Tris pH 7.5.

7. Add 1 ml 4M $MgCl_2$, allow to drain into column so that it has replaced previous solution then stopper column and leave for 15' at room temp.

8. Add more 4M $MgCl_2$ and collect 2 x 1 ml fractions as they drain from the column. Remove 10μl of each fraction for gel analysis.

9. Dialyse the combined fractions against dH_2O for 1 h, then against PBS overnight.

10. Remove antibody solution from dialysis tube. Concentrate if necessary and store in PBS plus 0.2% azide.

NOTE: If you wish to reuse the column to purify more antibody against the same protein, wash the column with excess equilibration buffer. This should return the bead/protein complex to a state where it can be reused.

Buffer B: 8 M Urea
0.1 M NaH_2PO_4
0.01 M Tris-HCl, pH 8.0
pH directly before use - 160μl 5M NaOH per 10ml

Buffer E: As B, but pH 4.5
pH directly before use – 11μl HCl per 10 ml

Equilibration buffer:

	100ml
150 mM NaCl	3 ml 5M
50 mM Tris-HCl, pH 7.4	2.5 ml 2M