MAXI-PURIFICATION OF INSOLUBLE HIS-TAGGED PROTEIN

THINGS YOU MUST KNOW FIRST:

- optimum protein induction conditions
- rough idea of protein yield – estimate by comparing to known amounts of BSA on a Coomassie stained gel and calculate mg protein per ml culture

Note: if you want to immobilize purified protein on CNBr supports, you must not include Tris in any of the buffers (use 200mM phosphate buffer instead of 100mM NaH_2PO_4 and 10mM Tris). You must also dialyse to get rid of the urea.

1 Qiagen Ni-agarose resin has a capacity of 5-10mg (varies from protein to protein) per 1ml resin (i.e 2ml of 50% slurry). Want to load resin at 80% capacity so that as many sites as possible are specifically bound. This will avoid non-specific binding of contaminants. The protocol uses 1ml of resin and thus you should aim to load 4-8mg protein.

2 Assuming protein expression conditions have been optimized, pellet an appropriate amount of *E. coli* culture containing HIS-tagged protein (i.e. amount that will yield 4-8mg protein). Store at –80°C.

3 Thaw cells on ice for 15'.

4 Add 8 ml **Buffer B**. (If protein does not solubilize in urea, can use Buffer A here instead). Stir at room temp for 15' – 1.5 h. Lysis is complete when the solution becomes translucent. Take 10µl aliquot for gel analysis. (GuHCl containing fractions need to be diluted 1:6 with H_2O before loading onto gel).

5 Spin slurry at 10,000 g for 30' and remove supernate. Take 10µl aliquot for gel analysis.

6 Mix protein supernate with 2 ml of 50% Ni-agarose slurry. Shake (200rpm) at room temp for ~1 h.

7 Decant protein/bead slurry into a column and collect flow through. Take 10µl aliquot for gel analysis.

8 Reload flow through and collect again. Take 10µl aliquot for gel analysis.

9 If cells were lysed in **Buffer A**, wash column twice with 8ml **Buffer B**. If cells were lysed in **Buffer B**, proceed directly to next step.

10 Wash column twice with 8ml **Buffer C**. Take 10µl aliquot of each wash for gel analysis.

11 Elute monomeric proteins with 4 x 1 ml **Buffer D**. Take 10µl aliquot of each for gel analysis.

12 Elute multimers/protein aggregates with 4 x 1 ml **Buffer E**. Take 10µl aliquot of each for gel analysis.

13 Store all fractions at –80°C until further use.

Buffer A:	6 M Guanidine HCl 0.1M NaH_2PO_4 0.01M Tris-HCl, pH 8.0	**50ml:** 28.65 g GuHCl 5ml 1M 0.25ml 2M Tris pH8
Buffer B:	8 M Urea 0.1M NaH_2PO_4 0.01M Tris-HCl, pH 8.0	**50ml:** 24g urea 5ml 1M 0.25ml 2M Tris pH8 25ml H_2O Add 160µl 5M NaOH per 10ml to adjust pH to 8.
Buffer C:	As B, but pH 6.3	**10ml non pHd B** plus 5µl 5M NaOH
Buffer D:	As B, but pH 5.9	**10ml non pHd B** plus 2.5µl HCl
Buffer E:	As B, but pH 4.5	**10ml non pHd B** plus 11µl HCl

pH solutions on day of use as dissociation of urea alters pH rapidly.