Purification of His-tagged proteins

Rapid Ni-NTA purification of proteins secreted into CHO cell media -

Upon harvesting media from cells I do the following:

0.2 uM filter the media and add NaAzide to 0.01%

Per 1L harvest

1. Add
100 ml glycerol
30 ml 5M NaCl
10 ml 1M Imidazole
50 ml Ni-NTA Superflow resin (100ml of material in suspension)

2. Gyro-rotary motion (150 rpm) room temp, 30-40 minutes. NO longer!

3. Decant into 250 ml conical bottom bottles.

4. Spin down beads 10' (room temp.) at 3750 rpm, Beckman GPR clinical centrifuge with gray conical bottom adapters

5. Assemble a large diameter yellow column with a 3-way stopcock.

6. Shut stopcock to no flow

7. Use motorized pipettor to remove 25 ml of supernatent, store in harvest bottles.

8. Use motorized pipettor to remove pelleted Ni-NTA beads from bottom of bottles,

9. Add these beads to the column.

10. Allow beads to settle in column

11. Repeat steps 4-10 to collect all beads

12. After beads have settled. Use motorized pipettor to remove excess media from the top of the column.

13. Wash column with (still at room temp -- 3-5 column volumes or until stable UV 280 nm absorbance is achieved):

50 mM Tris pH 8.0 (other buffers are ok)
300 mM NaCl
10% Glycerol
10 mM Imidazole (this is optional, and often not necessary)

(NaAzide may be added to this and all the subsequent buffers to increase their shelf life without effecting the Ni-NTA chromatography.)

14. Elute with

50 mM Tris pH 8.0 (other buffers are ok)
300 mM NaCl
10% Glycerol (this is optional, and often not necessary)
250 mM Imidazole

15. If the elution is done with glycerol and the column is to be used for only this protein the Ni-NTA resin may be regenerated with

2 column volumes

50 mM Tris pH 8.0 (other buffers are ok)
300 mM NaCl
10% Glycerol

2 column volumes

50 mM Tris pH 8.0 (other buffers are ok)
300 mM NaCl
10% Glycerol
50 mM NiCl2

and 2 column volumes

50 mM Tris pH 8.0 (other buffers are ok)
300 mM NaCl
10% Glycerol

Comments: This protocol does not require buffer exchange, scales to 5 L reasonably well (several centrifugations, with our equipment) and is fast, 1L may be purified in 2-3 hrs.

The method also works with media secreted from baculovirus harvests though not as well (50-80% of the yield achieved with buffer exchange.)

If the protein is being purified from baculovirus media (which is pH ~6.0) the following modification applies.

To the harvest add:

50 mM Tris pH 8.0 (other buffers are ok)
150 mM NaCl
10% Glycerol