# cFc Purification Protocol

1. Grow CHO cells and harvest supernatant
2. Filter media with 0.2M filter
3. Add 0.01% sodium azide (if purifying immediately, this is not necessary)
4. Buffer exchange CHO supernatant with 3-4L of: (see pg 31 for detailed protocol)
   50mM Tris pH 8.0
   300mM NaCl
5. Per 100mL of buffer-exchanged CHO sup, add:
   10 mL glycerol
   1 mL 1M imidazole
6. Prepare Ni resin:
   25mL 50mM Tris pH 8.0, 300mM NaCl, 10% glycerol (aka "buffer")
   25mL buffer + 50mM $NiCl_2$
   25mL buffer
7. Divide the buffer-exchanged CHO sup and Ni beads into 4 250mL conical bottom bottles
8. Nutate at room temperature for 1-1.5 hours
9. Allow the beads to settle in bottles by gravity for 5-10 minutes
10. Open the stopcock of the large diameter yellow column with a 3-way stopcock
11. Pour supernatant through column to quickly collect any beads that didn't settle (collect the FT and don't pour in settled beads until most of the sup from all 4 bottles has flowed through)
12. Add resin (which was at the bottom of the conicals) to column and allow the beads to settle
13. Set up the UV detector and chart recorder:
    UV detector sensitivity: 0.5
    Chart recorder rate: 2mm/min
14. Flow rate = 1ml/min
15. Zero UV detector with buffer
16. At room temperature, wash the column with 3-5 CV (or until a 280nm baseline is reached) of:
    50mM Tris pH 8.0
    300mM NaCl
    10% glycerol
    10mM imidazole
17. Elute with:
    50mM Tris pH 8.0
    300mM NaCl
    10% glycerol
    250mM imidazole
18. Run a gel on elution fractions or test absorbance by UV to determine which fractions to collect.
19. Dialyze Ni pool against 2x4L TBS overnight
    20mM Tris pH 7.4
    150mM NaCl
    2mM $CaCl_2$

20. Add 1/150 (w/w) ratio of Factor Xa to the dialyzed Ni pool. (NEB #P8010L)
21. Rotate at room temperature during the day then in the cold room overnight.
22. Spin out any precipitate
23. Add 10mM imidazole to cleavage reaction
24. Run a Ni column to remove any uncleaved Fc and His-tag fragments from cFc (cleaved Fc)
    - Bind to resin by slowly loading column with no peristalic pressure
    - Elute cFc with TBS + 10mM imidazole
    - Determine % of cleavage
    - Decide whether to elute uncleaved protein and cleave again
25. Concentrate cFc to ≤ 3mL with Amicon 10K/15mL concentrators
26. Run gel filtration column (Superdex 200 16/60) in TBS on the AKTA system
27. Concentrate to 25mg/mL with Amicon 10K/15mL concentrators