Adapted from Gelles Lab Protocol by Marc Ridilla and Linnea Lemma
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Dogic Lab Protocol for Purifying Kinesin Proteins

This protocol is for purifying His-tagged, truncated kinesin constructs using an AKTA.

Buffers
Wash Buffer:
50mM PIPES
4mM $MgCl_2$
50uM ATP
10mM βME (4^o)
20mM Imidazole

pH with KOH to 7.2

Elution Buffer:
50mM PIPES
4mM $MgCl_2$
50uM ATP
2mM DTT (4^o)
500mM Imidazole

pH with HCl to 7.2

Lysing
-Remove cells from freezer and put on ice
-add protease inhibitors to wash buffer to make lysis buffer
- 1 tablet/10mL of Pierce Protease Inhibitor Mini Tablet (Thermo #88665)
- a 1mg/mL of PMSF (MP Biomedicals, # 195381)
- add 1mg/mL of Lysozyme, Chicken, RZ3 Muramidase (US Biological # L9200)

-add 1-1.5x pellet volume of lysis buffer
-leave on ice to thaw
-resuspend and
-lyse cells using fine tip: 50% power, 50 % time, 15 minutes (Omni International Sonic Ruptor)
-every 5 minutes go in to re-support the falcon tube with ice (the lyser generates heat which melts the ice and can damage the proteins).
-centrifuge at >= 100,000 Gs for >= 30 min at 4^o to clarify lysate

Akta
-open software
-set pressure alarm (listed under column information)
-start flowing ethanol
-hook up column (Cobalt)—make sure there is no air in the system
-wash column with 5 column vol of ethanol, 5 col vol of water
-switch pumps to elution and wash buffers
-wash pumps

-wash column with 5 col vol elution buffer, 5 col vol lysis buffer. Do this at max speed possible within pressure limits.
-once there is a stable baseline in absorbance and conductivity – rezero

Cobalt Column—HiTrap TALON
-col vol is 5mL
-default flow rate is 5mL/min
-max flow rate is 20mL/min
-max pre-col pressure is 0.5MPa

Nickle Column—HisTrap HP
-col vol is 1mL
-default flow rate 1mL/min
-max flow rate 4.0mL/min
-max pre-column pressure 0.5MPa

Load the Protein
- remove the supernatant from centrifuge tubes
- while AKTA is in Manual load, use a syringe to load the clarified lysate into the injection loop (be careful to avoid bubbles!!!)
- AKTA setting for loading 5mL of protein
  1/5th of default flow rate
  Injection valve—inject mode
  5mL timer
-after the protein is loaded: run 15 col vol--or until there is a stable absorbance--of lysis buffer through the system up to default flow rate
-put eppendorfs in fraction collector
-change to manual load mode to not include the loop
-then elute at 1mL/min for 5 col vol: COLLECT FRACTIONS

Determining where the protein is and how much you have
- Mini Bradford: Determine which wells are blue—those are the one with protein which we should run on a gel and then combine and keep.
- Run a gel (SDS page, 8 or 12% agarose)

Concentrate and Store the protein
-use filter concentrators and swinging bucket centrifuge (3000G)
-blank with the stuff that come through the filter ("filtrate") and measure concentration using nano drop or other uv-vis spectrometer
-concentrate as desired
-store in 30% (final) sucrose or glycerol
-flash freeze in liquid nitrogen

Clean up Akta
(1) 5x column volumes of elution buffer over column
(2) Switch pumps to H20 and ethanol

(3) Wash pumps
(4) Change to inject mode to include loop
(5) 5x col vol of H20
(6) 5x col vol of ethanol