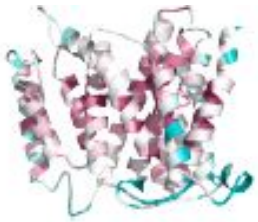
His Tag Purification

Purification Protocol



Theory and Introduction: Ni-Affinity Chromatography uses the ability of His to bind nickel. Six histadine amino acids at the end of a protein (either N or C terminus) is known as a 6X His tag. Nickel is bound to an agarose bead by chelation using nitroloacetic acid (NTA) beads. Several companies produce these beads as His Tagged proteins are some of the most used affinity tags in today's market. See handouts to Qiagen and Pharmacia, two commercial sources of NTA-Agarose resins. The general method is to batch absorb the protein onto the column, by mixing the beads with the sample, then pouring the slurry of NTA beads and protein into a column, where low concentrations of phosphate and imidazole are used to remove low affinity bound proteins. If needed, the imdazole can be increased to 20 mM before most His tagged proteins are eluted. Finally, higher concentrations of imidazole is used to elute the protein from the NTA-beads.

- Average binding capacity is about 5-10 mg of His tagged protein per ml of beads. Depending on many factors protein expression can range from 0.5 - 20 mg of His tagged protein per liter of medium.

Prepare Columns

- ***Preparation of Ni-Agarose Beads/Resin***:
  - Prepare 25 ml of beads by transferring 50 ml of a 50% slurry of beads equilibrate into a clean column. Wash and equilibrate the column by running 200 ml of His Elution Buffer followed by 500 ml of His Binding Buffer through the column. This SHOULD be done ahead of time! Store prepared beads with a few ml of His Binding buffer at RT.

1. Prepare slurry and beads (already done)

Prepare the cell lysate (freezing and heating)

1. Make sample tubes for each of your samples with 400ul TEG (Tris EDTA Glucose)
2. Add 250 ul of sample to each new tube with TEG
3. Place in -20C freezer on side for 15min to freeze
4. Remove from freezer and add place in 37C water bath until thawed
5. Repeat 5-6 times of heating and freezing cycles
6. Centrifuge for 10min
7. Collect 500ul of supernatant (lysate) to be used in Purification step

Column Purification:

- **Purification**:
  - Save 100 ul of lysate. Add clarified lysate (if frozen, check for ppt material. If there is any clumpy or ppt material or if the lysate is cloudy, centrifuge and keep the supernatant) to the washed beads.
  - *Batch Binding* - if protein expression is low or the His-tagged protein binds with low affinity then use a batch purification method.
    - Combine the washed beads and lysate onto a drained and capped column or a 50 ml falcon tube for smaller volumes. Use a spatula and/or a transfer pipette to suspend the beads. Tightly cover with parafilm and incubate rocker for 30 min at room temp.
    - Replace the column on the stand and allow most of the beads settle then open column. Add frit back to column. ***Reapply the flow thru***. This is the non-binding protein. Continue with purification
  - *Column Binding* - Flow the clarified lysate through the column. Save the eluate as flow-through in one fraction. If there is a concern with binding efficiency the flow-through can be reapplied. Continue with the purification.

Washing:
After binding:
Wash beads with 1ml of Ni-Column His Binding Buffer, save flow through wash in one fraction(tube)
Wash the column with 2ml of His Wash buffer (to remove weakly bound proteins)

Elution: (collecting Samples)
Elute the protein with 500ul of His Elution Buffer (so this 10times) keep each fraction in separate tube for analysis in SDS PAGE (label tubes 1-10 and place in gel in that order)

Let tubes sit at room temperature overnight or in 4C for two days

Analyze using 12% SDS Page