Protocol

GoldBio His-Tag Column Prep Protocol

Pouring and Packing an Agarose Column

Introduction

Purification of His-Tagged proteins is a fundamental protein purification procedure that involves the expression of proteins containing histidine residues. During protein isolation, these histidine tags can bind to metal ions, allowing for separation of proteins. This method of tagging proteins is particularly useful in the process of purifying denatured proteins (denaturing conditions). However, this procedure can also be used with proteins isolated from their natural environments (native conditions). His-tag purification can achieve purities of up to 95%, while different metal ions offer the ability to select specific binding capabilities. This protocol describes a complete his-tag procedure including column creation and column equilibration, the running of the column, and elution of the tagged proteins.

Materials

- Bulk affinity resin
- DI-H_2O
- Phosphate Buffer, pH 8 (GoldBio Catalog # P-500)
- His-Tag Buffer Set (GoldBio Catalog # I-906), containing:
    1. Wash buffer
    2. Elution buffer
- Plastic column with bottom and top stoppers (GoldBio Catalog # P-301)
- A beaker or tube to catch flow through
- A ring-stand and clamp
- Small funnel for head of column

Method

1. Degas the agarose bead resin and buffer completely prior to adding anything into the column.

2. Add one column volume of wash buffer to the empty column and slowly drip buffer through the column to remove air bubbles from under the frit.

> **Note: If air bubbles remain, tap the column to remove them. Then, if air bubbles persist, the column can be centrifuged at a low rpm to remove them.**

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3. Gently shake the bottle to obtain a homogenous suspension of Affinity Agarose Beads (Ni, Co, Cu, Zn or Metal Free). Place a sterile funnel in the head of the column, then open the bottom cap to start the flow of buffer through the column.

4. Slowly run the agarose bead suspension down the walls of the column.

5. Run the column and continue to pour the suspension into the column until the desired column bed height is reached.

   Note: Do not allow the beads to completely settle in the column, the best columns are made from a continuous pour. If you inadvertently allow the beads to settle, use a pipette to mix the top of the settled matrix, then continue to add new agarose beads to the column.

   Note: Do not allow the column to run dry. If the column runs dry, you must re-pour the column. If you are using a flow adapter, insert the adapter into the column head until it begins to displace the liquid and ensure that no air is trapped under the sintered disc of the flow adapter.

6. Wash the preservative off the resin by adding 5-10 column volumes of buffer or DI-H_2O and running it through the column, again making sure the column doesn't run dry.

7. If using the column immediately, move on to equilibrating the column. If storing the column, add a little less than one column volume buffer or Di-H_2O, place the bottom and top caps, and store upright at 4°C. To prevent microbial growth, you can add sodium azide to a final concentration of 0.02%.

Equilibrating the Column

1. Equilibrate the column by adding 5 to 10 column volumes of Wash buffer. Make sure to degas all the solutions before adding to the column to avoid the formation of bubbles.

Running the Column

1. Load the lysate, containing the solubilized 6x histidine labeled protein, onto the column. Control the flow rate of the addition of the lysate. GoldBio recommends a binding flow rate of 12 ml/hour for a 1-ml column. Alternately you may want to load the column with one third of the column volume and allow the material to bind for five minutes prior to adding the next load of lysate.

2. Wash the column with 10 column volumes of wash buffer. The flow rate for a 1-ml column can be 30 ml per hour. Avoid compressing the agarose beads. Do not compress them by applying too much pressure via a pump or a large pressure head.

3. Elute the protein using one of the following methods:
    a. Apply a linear gradient of 10mM imidazole to 50mM imidazole in phosphate buffer, pH 8 (GoldBio Catalog # P-500).
    b. Use a step gradient of elution buffer (i.e. elute with 300mM, 400mM and 500mM imidazole in elution buffer)
        i. For step gradient, dilute elution buffer with phosphate buffer, pH 8
            - 100mM (50 ml) = 10 ml elution buffer: 40 ml phosphate buffer
            - 200mM (50 ml) = 20 ml elution buffer: 30 ml phosphate buffer
            - 300mM (50 ml) = 30 ml elution buffer: 20 ml phosphate buffer
            - 400mM (50 ml) = 40 ml elution buffer: 10 ml phosphate buffer

Note: Regardless of which method you use for elution you should collect fractions that are commensurate with the column bed volume. For example, if you are using a 1 ml bed column, we recommend that you collect 200 µl fractions for assay. The protein should theoretically elute in the included volume of the column which is approximately equivalent to two thirds of the column bed volume.

Tips

- To prevent aggregation (nonspecific binding of the target protein with His residues in adjacent proteins), add 10mM 2-mercaptoethanol to loading, washing, and elution buffers.
- Addition of Triton X-100 or Tween-20 to the elution buffer might reduce disulfide bonds with other proteins.
- Salt and imidazole concentrations might require optimization for the specific target protein.
- To prevent nonspecific hydrophobic interactions, add salt (500mM maximum), glycerol (20% maximum), or ethanol (20% maximum) during elution.
- Buffer components might require optimization for the specific target protein.
- Perform cell lysis during sample preparation in a buffered solution, pH 8.0.
- Consider saving the flow-through from washing and binding steps since they might contain your target protein, if the target protein did not efficiently bind the resin.
- Perform column packing and purification procedures in a cold room or at 4°C to prevent denaturing of the protein.

Associated Products

- Cobalt Agarose Beads, High Density (GoldBio Catalog # H-310)
- Copper Agarose Beads, Low Density (GoldBio Catalog # H-311)
- Nickel Agarose Beads, High or Low Density (GoldBio Catalog # H-320 or # H-321)

- Zinc Agarose Beads (GoldBio Catalog # H-330 or # H-331)
- Metal Free Agarose Beads (GoldBio Catalog # H-300 or # H-301)

References

Arnau, J., Lauritzen, C., Petersen, G. E., & Pedersen, J. (2011). Reprint of: Current strategies for the use of affinity tags and tag removal for the purification of recombinant proteins. *Protein Expression and Purification*. Doi:10.1016/j.pep.2011.08.013.

Bornhorst, J. A., & Falke, J. J. (2000). Purification of proteins using polyhistidine affinity tags. *Methods in Enzymology Applications of Chimeric Genes and Hybrid Proteins Part A: Gene Expression and Protein Purification,* 245-254. Doi:10.1016/s0076-6879(00)26058-8.