Simple His-Tag Column Prep Procedure Version 2

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Abstract

GoldBio's simple His-Tag column prep protocol is a quick and easy method to pouring and packing an agarose resin column.

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Materials

5X Phosphate Buffer, pH 8 P-500 by Gold Biotechnology

His-Tag Buffer Set I-906 by Gold Biotechnology

Plastic Columns P-301 by Gold Biotechnology

Protocol

Step 1.

Necessary Supplies

- Bulk affinity resin
- Di-H_2O
- Phosphate Buffer, pH 8 (GoldBio Catalog # P-500)
- His-Tag Buffer Set (GoldBio Catalog # I-906)
- Empty plastic column with bottom and top stoppers
- A beaker or tube to catch flow through
- A ring stand and clamp
- Small funnel for head of column

Prep the Column

Step 2.

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

Step 3.

Add one column volume of **Wash Buffer** to the empty column and slowly drip buffer through the column to remove air bubbles from under frit. If air bubbles remain, tap the column to remove them. If air bubbles remain, the column can be centrifuged at a low RPM to remove them.

Step 4.

Gently shake the bottle to obtain a homogenous suspension of Affinity Agarose Beads (Ni, Co, Cu, Zn or Metal Free). Place a funnel in the head of column, then open the bottom cap to start the flow of buffer through the column. Slowly run the Agarose bead suspension down the walls of the column. Run the column and continue to pour the suspension into the column until the desired column bed height is reached. 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. 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 be sure that no air is trapped under the sintered disc of the flow adapter.

Step 5.

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.

Step 6.

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

Step 7.

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

Running the Column

Step 8.

Load the lysate (which contains the solubilized 6x Histidine labeled protein) onto the column. Control the flow rate of the addition of the lysate. We recommend a binding flow rate of 12 ml per hour for a one 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.

Step 9.

Wash the column with 10 column volumes of Wash Buffer. The flow rate for a one ml column can be 30 ml per hour. Avoid compressing the Agarose beads. Do not compress the Agarose beads by applying too much pressure via a pump or a large pressure head.

Step 10.

Elute the protein using one of the following methods:

a. Apply a linear gradient of 10mM Imidazole to 500mM Imidazole in Phosphate Buffer, pH 8; or

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 : 40 ml Phosphate
- 200mM (50 ml) = 20 ml Elution : 30 ml Phosphate
- 300mM (50 ml) = 30 ml Elution: 20 ml Phosphate
- 400mM (50 ml) = 40 ml Elution: 10 ml Phosphate

NOTES

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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 one 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.