Low Density Zinc Resin

Catalogue No.:abx291330

Zinc chelates generally recognize two exposed histidines in vicinal position. Low Density Zinc has a lower amount of available groups that usually improve the selectivity/specificity. This resin is recommended working with proteins that are difficult to separate, and is suitable for use in batch or column purifications (Low Flow).

Bead Geometry & Size	Spherical, Standard: ~ 50-150 µm
Crosslinked	Yes
Agarose %	6%
Matrix	Stable in all commonly used reagents
Binding/Loading Capacity (µmol Me^{2+}/ml gel)	5-19
Antimicrobial Agent	20% Ethanol

Target: Low Density Zinc Resin

Storage: Store between 2-8 °C. Do not freeze.

Note: THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC, THERAPEUTIC OR COSMETIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Abbexa LTD, Cambridge, UK · Phone: +44 (0) 1223 755950 · Fax: +44 (0) 1223 755951
Abbexa LLC, Houston, TX USA · Phone: +1 832 327 7413
Abbexa BV, Leiden, NL
Website: www.abbexa.com · Email: info@abbexa.com

Directions for use:

Suggested Procedure

The following procedure is for purification of His-tagged proteins under native conditions. The strength of the binding of the protein to the resin will depend on: the resin employed (both the number of chelate groups and the chelant metal), the accessibility of the His-tag, the pH, and the buffer composition.

1. Shake the bottle to obtain a homogenous suspension. It is recommended to de-gas all solutions before adding them to the column to avoid formation of bubbles.
2. Place a funnel in the head of the column and slowly run the suspension down the walls of the column. Avoid bubbles. Repeat until the desired column height is obtained.
3. Insert the adapter gently in the column head until it begins to displace the liquid. Ensure that no air is trapped under the net.
4. Connect the pump to the column and watch that the column height remains the same as the flow of distilled water is passing through.
5. When a constant height has been obtained, maintain the flow with the addition of 5 column volumes of distilled water to completely eliminate the preservative.
6. Equilibrate the column with 5-10 column volumes of binding buffer. Recommended conditions: Linear Flow Rate: 26 cm/h; Recommended Flow Rate: 0.5-1.0 ml/min; Maximum Pressure: 2.6 psi (0.18 bar).
7. Once the resin is equilibrated, the sample containing the fused protein for purification can be applied. In some cases a slight increase of contact time may facilitate binding. Note: Binding capacity can be affected by several factors, such as sample concentration, binding buffer or the flow rate during sample application.
8. Wash the resin with binding buffer until the $OD_{280\ nm}$ reaches the baseline level.
9. The purified protein can be eluted via several methods:
   - Add a competitive ligand such as 0.5 M imidazole (a concentration gradient of 0-0.5 M can also be used; most proteins are eluted in concentrations around 250 mM). Other reagents that can be used as competitive ligands are histidine and ammonium chloride. Generally, eliminating imidazole itself afterwards is not necessary, but if it is, it can be done by dialysis, precipitation with ammonium sulphate, or ultrafiltration.
   - Reduce the pH (with or without gradient) to between pH 3.0 and 4.0.
   - Use reagents such as 0.05 M EDTA or EGTA, which will also cause elution of the chelating metal.

Notes:

- In some cases, an excessively drastic elution can be resolved through the use of other activated beads (another chelate cation with lower affinity or fewer groups), where the interaction is weaker.
- For most applications, it is not necessary to eliminate the His-tag. However, this elimination is necessary for certain applications such as X-ray crystallography or RMN, where the protein structure is to be determined later. For these purposes, a His-tag is usually spliced to the protein, at a protease cleavage site.
- Please note that sometimes agarose beads can aggregate, and it is normal to see beads clumped together. This phenomenon does not cause any issue with the resin and can be solved by stirring the suspension for ~24 h.

Regeneration Procedure

During the life of the resin, it may lose binding points because some protein is retained. A loss of the binding capacity may therefore be observed in successive cycles. To return to the starting state, regeneration may be necessary. Regeneration consists of the complete elimination of the metal and therefore of the retained protein. In general, column regeneration is always necessary when changing proteins. When continuing with the same protein, it is recommended to do a regeneration when an appreciable diminution in the yield is observed. The frequency of these stages varies with the protein and the conditions used.

1. Eliminate the metal from the resin by washing the column with 5 column volumes of: 20 mM sodium phosphate, pH 7.0, containing 0.5 M NaCl, 50 mM EDTA.
2. Eliminate excess EDTA by washing the column with 5 column volumes of distilled water.
3. If the sample contains denatured proteins or lipids:
   1. Eliminate ionic interactions: Wash for approximately 20 minutes in a solution containing 1.5 M NaCl, then wash with 10 column volumes of distilled water to eliminate ions.
   2. Eliminate precipitated proteins (may be responsible for column pressure changes): Wash for at least 2 hours with a solution containing 1.0 M NaOH. Eliminate the NaOH with 10 column volumes of distilled water.
   3. Eliminate strong hydrophobic interactions: Resuspend the resin using a solution of 30% isopropanol for approximately 20 minutes. Wash with 10 column volumes of distilled water to eliminate the isopropanol.
   4. Wash for 2 hours with a solution 0.1 M acetic acid containing 0.5% of non-ionic detergent. Rinse with 70% ethanol (approximately 10 column volumes) to remove the detergent. Finally wash with 10 column volumes of distilled water to remove the ethanol.
4. Load the column with the corresponding metal: once the excess EDTA has been eliminated, add 5 volumes of the 0.1 M metal solution (normally chlorides or sulphates are used).

General Recommendations

• The choice of binding buffer depends on the particular properties of the protein as well as of the type of chelate used. The buffers used most frequently are acetate (50 mM) or phosphate (10-150 mM). The pH of binding buffers is generally in the neutral region (pH 7.0-8.0), though can vary over the range 5.5-8.5. To avoid ionic interchange, add 0.15 - 0.5 M of NaCl. Buffers should be degassed before use to avoid the formation of bubbles. In some cases, to increase the selectivity of the binding of target protein, it is necessary to add to the binding buffer a small concentration of imidazole (10-40 mM). It is important to use high purity imidazole to avoid affecting the $OD_{280\ nm}$. It is important to avoid the presence of agents like EDTA or citrate at all times.

• Recombinant proteins often form insoluble inclusion bodies. If so, these need to be rendered soluble by a purification under denaturing conditions (e.g. 8 M Urea or 6 M Guanidine-HCl).

• The resin is compatible with the following reducing agents: 10 mM reduced glutathione; 20 mM beta-mercaptoethanol; 5 mM DTE; 5 mM DTT.

• The resin is compatible with the following detergents: 2% Triton X-100; 2% Tween-20; 1% CHAPS.

• This resin is stable in the following buffers and additives: 50 mM Na_2HPO_4, pH 7.5; 100 mM Tris-HCl, pH 7.5; 100 mM Tris-Acetate, pH 7.5; 100 mM HEPES, pH 7.5; 100 mM MOPS, pH 7.5; 0.1 M sodium acetate, pH 4.0; 0.01 M HCl; 0.1 M NaOH; 1 M NaOH; 30% 2-propanol; 70% HAc; 2% SDS; 20% ethanol; 20% ethanol and 50% glycerol; 2 M imidazole; 100 mM Na_2SO_4; 1 mM EDTA and 10 mM $MgCl_2$; 60 mM citrate; 60 mM citrate and 80 mM $MgCl_2$.

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