Co-NTA Resin Protocol

The procedure outlined below is recommended as a starting point for purifications using this resin. All procedures and buffer formulations may be optimized by the user based on their own specific antibody samples and experiences.

Buffer Preparation

All water and buffers are recommended to be filtered with a 0.22 μm or 0.45 μm filter prior to use.

Equilibration buffer: 0.02M PB, 0.5M NaCl, adjust the pH to 7.4

Elution buffer: 0.02M PB, 0.5M NaCl, 0.5M imidazole, adjust the pH to 7.4

Wash Buffer: 0.02M PB, 0.5M NaCl, 0.02-0.04M imidazole, adjust the pH to 7.4

Pretreatment of the column

1. The packing must be washed with 5-10 times the column volume of purified water at 0.5 mL/min (1 mL columns) or 2.0 mL/min (5 mL columns).
2. Equilibrate with 5-10 times the column volume of equilibration solution at 0.5 mL/min (1 mL column) or 2.0 mL/min (5 mL column) until the baseline is stable.

Sample Handling

1. Samples are subjected to centrifugation or filtration prior to sampling.

Sample Purification

1. Sample uptake: The volume of sample uptake is determined based on the amount of substance in the sample and the binding load of Co-NTA.
2. Wash: The column is to be washed with equilibrium buffer until the UV absorption is close to the baseline.
3. Competitive elution: this is achieved by linearly or gradually increasing the concentration of substances with affinity for metal ions, such as 0~2M NH4Cl, 0~0.5M imidazole, 0~0.5M histidine. Gradient elution is better performed at a constant pH of the equilibration buffer.
4. Lowering the pH of the elution buffer: as the pH is lowered, the weakly and strongly bound proteins are eluted sequentially. When the pH is lowered below 4, the metal ions will dissociate with the medium to achieve the desired elution effect. (If the target protein is sensitive to low pH, it is recommended to add 1/10 volume of 1M Tris-HCl, pH 9.0, to the elution collection solution for neutralization.) A 0.05 M chelating agent, such as EGTA or EDTA, can dissociate the metal ions from the medium and can also be used to elute denatured or precipitated proteins. The Co^{2+} in the eluted product can be removed by a desalting column, and the medium can be re-saturated with 0.2M CoSO4.

In Situ Cleaning

1. As the number of uses of the chromatography medium increases, so too does the accumulation of pollutants in the column. Regular in-situ cleaning has been shown to be an effective method of preventing the accumulation of pollutants and maintaining the stable working condition of the chromatography medium. The frequency of in-situ cleaning is determined by the degree of contamination of the medium during use. In cases where contamination is more severe, it is recommended to carry out in-situ cleaning after each use to ensure the reproducibility of results. The recommended cleaning conditions for different types of impurities and contaminants are as follows:
2. Cobalt ions are first removed.
3. Removal of proteins adsorbed by ion exchange: The column should be washed with 2 to 3 column volumes of 2M NaCl solution, followed by a wash with 3 column volumes of distilled water.
4. Removal of precipitated or denatured substances: treat with 1M NaOH for 0.5~1h.
5. Removal of hydrophobically bound substances: The column should be washed with 2 column volumes of 70% ethanol or 30% isopropanol, and then immediately reversed with at least 5 column volumes of sterile equilibration buffer.

Washing

1. The protein samples should be centrifuged and filtered before being loaded onto the column, to avoid solids from accumulating on the column, which will affect the service life of the column.
2. After each use of the column, there will be some proteins, lipids and other substances left on the column, and the more it is used, the more it accumulates, which will have an impact on the performance of the column (loading, mobility, column efficiency); this is very much related to the degree of cleanliness of the samples; it is recommended that a cleaning can be carried out after 2-3 times of use, in order to remove the residues on the column.
3. Wash 5-10 column volumes with 30% isopropyl alcohol for 10-15 min contacting, followed by 10 times the column volume of purified water.
4. Wash with 1.5M NaCl solution for 8-10 min, followed by a 10-fold column volume wash with purified water, and finally rinse with 20% ethanol for 5-10 times the column volume to preserve it.

Column Regeneration

1. It is recommended that the packing be regenerated after every 5-10 uses, depending on the cleanliness of the initial sample being purified:
2. The column should be rinsed with 5-10 times the column volume of purified water;
3. The column should be rinsed with 5-10 times the column volume of 0.02 M Tris-HCl, 0.1 M EDTA pH 8.0, then 5-10 times the column volume of purified water;
4. The column should be rinsed with 5-10 times the column volume of 1. 0 M NaOH, followed by a 10-15 minute period of undisturbed standing, and subsequent rinsing with purified water to achieve neutrality;
5. The column should be rinsed with 5-10 times the column volume of 0.1 M $NiSO_4 \cdot 6H_2O$, followed by 0.5 h of undisturbed standing, and then rinsing with 5-10 times the column volume of purified water;
6. The column should be rinsed with 5-10 times the column volume of 20% ethanol, and then store the solution.

Precautions

1. The product should be sealed and stored in 2-8℃(preservation solution is 20% ethanol), ventilated, dry and clean place. Do not be frozen.
2. Store the used columns at 4℃ (preservation solution is 20% ethanol); avoid contact with oxidizing agents; avoid prolonged exposure to pH < 4 (7 days, 20℃).
3. Valid for 3 years.