

Ni-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: 20 mM Na₂HPO₄, 0.5M NaCl, pH 7.4

Binding buffer: 20 mM Na₂HPO₄, 0.5M NaCl, 20-40 mM imidazole, pH 7.4

Elution Buffer: 20 mM Na₂HPO₄, 0.5 M NaCl, 500 mM imidazole, pH 7.4

Sample preparation

Protein samples should be centrifuged and filtered before being loaded on the column to avoid the accumulation of solids on the column, which will affect the life of the column.

Purification Procedure:

1. Perform all steps at a flow rate of 0.5ml/min for a 1ml column, or 2.0ml/min for a 5ml column.
2. Wash the resin with 5-10 column volumes (CV) of distilled water.
3. Equilibrate the column with 5-10 CV Equilibration Buffer until the baseline is stable.
4. Load the sample at the flow rate outlined in Step 1.
5. After loading, wash the column with Equilibration Buffer until the baseline is stable (OD280 is at baseline).
6. Wash the column with 5-10 CV of Binding Buffer. Collect the unbound sample.
7. Elute the target protein with 5-10 CV Elution Buffer. Collect the elution sample.

Resin Cleaning

After each use of the column, proteins, lipids and other substances will remain on the column. Accumulation of these residues will affect the performance of the column (capacity, fluidity, column efficiency). This is directly related to the cleanliness of the samples loaded.

It is recommended to perform a thorough column cleaning after every 2-3 uses to remove the residue on the column. There are two methods to clean the column.

Cleaning Procedure 1:

1. Wash with 5-10 CV of 30% isopropanol, ensuring 10-15 min contact time.
2. Wash with 10 CV of distilled water.
3. Equilibrate with 5-10 CV of 1X PBS with 20% ethanol before storage.

Cleaning Procedure 2:

1. Wash with 1.5M NaCl for 8-10min.
2. Wash with 10 CV of distilled water.
3. Equilibrate with 5-10 CV of 1X PBS with 20% ethanol before storage.

Precautions

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