Ni-TED 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.

Binding 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, 20mM imidazole, adjust the pH to 7.4.

Aqueous buffer, 0.01M NaOH, 0.01M HCl (1 week); 10 mM EDTA, 5 mM DTT, 5 mM TCEP, 20 mM β-mercaptoethanol, 1 M NaOH, 6 M guanidine hydrochloride (24 hours); 500 mM imidazole, 100 mM EDTA (2 hours); 30% isopropyl alcohol (20 minutes)

Protocol (1 mL column mounted resin is used as an example)

1. Sample preparation: Remove host cell debris by centrifugation, etc., then pass through a 0.45 μm microporous filter membrane and dilute appropriately with Binding Buffer. For maximum loading, do not add imidazole to the protein sample solution.
2. Water wash: Wash the resin with 5-10 column volume of purified water at 50-150 cm/h to remove ethanol.
3. Equilibrate: Equilibrate the medium with 5-10 column volume of Binding Buffer at 150-600 cm/h to ensure that the components and pH match the sample.
4. Sampling: The sample is centrifuged and filtered (0.45 μm) and then sampled at a low flow rate, 150 cm/h is recommended. (Note: The binding capacity of proteins varies with the type of lysate, target protein, flow rate and pH; The lower the flow rate, the better the binding. The flow rate can be reduced if the temperature during purification is low to prevent column pressure from becoming too high due to the viscosity of the sample and buffer).
5. Wash: Use 10-20 column volume of Wash Buffer at 150 cm/h to remove the non-specifically adsorbed heteroproteins and collect the elution solution for later analysis.
6. Elution: Elute with 5-10 column volume of Elution Buffer at a low flow rate and collect the eluent. (Note: There are alternatives to imidazole for elution, such as lowering the pH (2.5-5.0). This product does not require regeneration with Ni2+ after each use, but does regenerate if a low pH is used).
7. Water Wash: Wash the medium with 5-10 column volume of purified water at 0.5 mL/min to remove eluent from the medium.
8. Storage: Wash the medium with 5-10 column volume of 20% ethanol at 0.5 mL/min and store at 2-8°C.
9. Regeneration: Wash the medium with 2 column volume of 1 mol/L NaOH, followed by a rinse with 10-20 column volume of purified water. If inactivated proteins or lipids cannot be washed out during regeneration, they can be removed by Cleaning In Place (CIP).

Cleaning In Place

1. For highly bound proteins or lipids, the following procedure may be used for cleaning: 2 column volume of 1 mol/L NaOH, 2 column volume of 4 mol/L urea or 3 mol/L guanidine hydrochloride, 2 column volume of 70% ethanol or 30% isopropanol, 5-10 column volume of purified water, and 5-10 column volume of equilibration buffer
2. It is recommended to wash the resin every 5-10 uses, depending on the cleanliness of the starting sample to be purified.

Note

1. Water and chemical reagents used to prepare the buffer must be of high purity and passed through a 0.45 μm microporous membrane prior to use. The addition of imidazole to the sample and binding buffer is not recommended. The optimal concentration of imidazole in the wash buffer depends on the protein sample, 10-20 mM imidazole is generally used.
2. NaCl in the buffer is used to inhibit ion exchange of the resin.
3. In general, the concentration of imidazole in the elution buffer should elute most of the target protein, and it is possible to increase the concentration of imidazole and the incubation time to make the elution better.
4. In case of inclusion body purification, adding 8 M urea or 6 M guanidine hydrochloride to the equilibrium solution, wash buffer and elution buffer is recommended.

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.