His-tag Protein Purification Resin (HP-TED) Product Manual

1. Product Introduction

His-tag protein purification resin is suitable for the purification of His-tag fusion proteins. This product can be used to quickly extract His-tag fusion proteins from cell culture broth.

His-tag protein purification resin (HP-TED) uses polyacrylate microspheres as the matrix and ethylenediaminetriacetic acid (TED) as the metal chelating ligand. Ni^{2+} can be stably chelated even in solutions containing a certain amount of EDTA, will not easily leach; meanwhile, the product does not need to be re-chelated with Ni after CIP, and can be cleaned directly with NaOH solution. This product can significantly save sample pre-processing preparation and post-processing operation time and is especially suitable for the purification of His-tag proteins expressed and secreted by eukaryotic cells such as insect cells and mammalian cells.

It conventionally chelates Ni^{2+}, but can also be customized according to customer requirements, chelating Cu^{2+}, Co^{2+}, Zn^{2+} and other ionic groups.

2. Product Properties

Parameter	Technical Specification
Average particle size	70±20 μm
Pore size	30 nm
Matrix beads	Polyacrylate microspheres
Ligand	TED
Dynamic binding capacity	≥20 mg His-Pro/ml wet gel
Pressure upper limit	1 MPa
Chemical stability	Resistant to 10 mM EDTA, 0.5M NaOH, and 6M Guanidine HCl for 24 hours; resistant to 500 mM imidazole, 100 mM EDTA for 2 hours
pH stability	3-13 (long-term); 2-14 (short-term)
Storage	4-30 ℃ (20% ethanol)

3. Operation Steps

The process of separating and purifying His-fusion proteins using His-tag protein purification resin usually includes steps such as equilibration, loading, washing, and elution.

Equilibration: Equilibrate the chromatography column with 5-10 CVs of equilibration buffer until the conductivity and pH of the effluent remain stable (consistent with the equilibration solution). In actual operation, a neutral/weakly basic (pH 7~8) high-salt (0.15-1.0 M NaCl or other neutral salt) buffer is generally used. Among them, phosphate buffer system is commonly used, such as 20 mM PB+0.5 M NaCl, pH 7.4. For strongly binding his-tag proteins, low concentrations (20~40 mM) of imidazole can be added to the equilibration buffer.

Loading: The buffer of buffer should be as consistent as possible with the equilibration solution. Solid samples can be prepared by dissolving in equilibration solution; low-concentration sample solutions can be dialyzed with equilibration solution; high-concentration sample solutions can be diluted with equilibrium solution. To avoid clogging the column, samples should be centrifuged or micro-filtrated. The loading amount is calculated based on the binding capacity of the resin and the target protein content in the feed. To reduce the adsorption of impurity proteins on the chromatography column, the imidazole in the sample buffer can be appropriately increased while ensuring the adsorption of the target protein. For proteins as inclusion body, 8M urea or 6M guanidine hydrochloride can be added to the equilibration, loading, and elution buffers accordingly.

Washing: After loading the sample, continue to wash to the baseline with equilibration buffer.

Elution: There are generally two ways of elution. One is to use competitive reagents, such as imidazole (0-0.5 M), histidine (0-0.05 M), ammonium chloride (0-2 M), to remove the protein from the column. The second is to reduce the pH value and elute the target protein. Most proteins can be eluted in the range of pH 4-6. If you use the competitive reagent imidazole or reduce the pH value to elute the protein, the metal ions will still be bound to the column; if you use the competitive reagent histidine or ammonium chloride to elute the protein, the complex of the metal ions and the protein will be eluted together.

0.15-1.0 M NaCl must be added to the elution buffer to eliminate ion exchange. For gradient elution, it should be performed at a constant pH of the starting equilibration solution, linear gradient or step gradient elution can be used.

Regeneration: After the resin has been used several times (the actual number of uses is related to the characteristics of the culture broth and impurity), or before chelating other metal ions, it needs to be regenerated.

(1) This product can withstand a certain concentration of NaOH solution. It can be directly cleaned with NaOH solution without Ni removal and regeneration.

Use 5-10 CVs of 0.1 M NaOH cleaning agent to remove pigments or other strongly adsorbed proteins, and then wash with 10-20 CVs of equilibration buffer or deionized water to remove residual solution.

(2) When the chelated metal ions need to be replaced, the ion currently chelated must be removed and resin regenerated.

a. Remove Ni:

Wash the resin with 5 CVs of EDTA solution (0.2 M EDTA + 0.5 M NaCl, pH 7.0) at a low flow rate (recommended 1/2 of the normal loading flow rate), then wash with 5 CVs of 0.5M NaCl to remove residual EDTA.

b. Chelate Ni:

Mix the resin with 3-5 CVs 0.1 M $NiSO_4$ solution for 4 hours, then filter. (You can also perform on-column cleaning by loading 3-5 CVs 0.1M $NiSO_4$ solution onto the column at a low flow rate. To ensure sufficient Ni chelation, you can load the $NiSO_4$ solution onto the column for three cycles).

c. Equilibrate:

Wash the resin with 3 CVs of deionized water, followed by 5-10 CVs 0.25M imidazole + 0.5M NaCl solution to remove residual Ni^{2+}, and finally wash the resin with 5 CVs deionized water.

Storage: Use 2-5 CVs 20% ethanol to clean the resin and store it in an environment of 4-30°C after campaign.

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