His-tag Protein Purification Resin (HP-IDA) 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-IDA) uses polyacrylate microspheres as the matrix and iminodiacetic acid (IDA) as the metal chelating ligand. It conventionally chelates Ni^{2+} (often called nickel resin), 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	IDA
Dynamic binding capacity	≥30 mg His-Pro/ml wet gel
Pressure upper limit	1 MPa
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-tag proteins using His-tag protein purification resin usually includes steps such as equilibration, loading, washing, elution, and regeneration.

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. In order 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. 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.

2. Wash:

Wash with > 2-3CVs 2 M NaCl to remove ionically bound proteins, and then wash with >3CVs pure water.

Wash the resin with 5 CVs 0.5-1 M NaOH (or 2 M NaCl solution) to remove pigments or other strongly adsorbed proteins, then equilibrate and wash with 0.2 M PBS buffer for 5-10 CVs (the pH of the solution can be detected by pH if possible, and wash until the pH value is consistent with buffer solution).

It can be washed with 5-10 CVs of 70% ethanol or 30% isopropyl alcohol (>20 minutes), and wash with 3-10 CVs of pure water. **Please note that it is not easy to wash with isopropyl alcohol, so it is recommended to increase the washing volume.**

Alternatively, 2 CVs of a basic or acidic solution containing detergent may be used. For example, use 0.1-0.5% non-ionic detergent + 0.1 M acetic acid to wash for 1-2 hours, then wash with 5-10 CVs of 70% ethanol to remove the detergent, and rinse with 3-10 CVs of pure water.

3. 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 chelating by loading 3-5 CVs 0.1 M $NiSO_4$ 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).

4. Equilibrate:

Wash the resin with 3 CVs of deionized water, followed by 5-10 CVs 0.25 M imidazole + 0.5 M 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.

Duoning Biotechnology Group

marketing@duoningbio.com

www.duoningbio.com/en

6/F manulife place 348kwun tong road kl, HongKong, PRC.

2024 Ver.01