Product Data Sheet

Seplife® 6AG NTA/Ni/90

1. Description

Seplife® 6AG NTA/Ni/90 is a metal affinity chromatography resin based on agarose functionalized with nitrilotriacetic acid (NTA) and loaded with Ni showing high affinity for his-tagged proteins.

- Nickel loaded for high specific binding with Histidine tag on recombinant proteins.
- Suitable for small scale and large scale purifications of His-tagged proteins from other proteins in the feed.
- Seplife® 6AG NTA/Ni/90 resin is suitable for the purification of His-tag proteins under native or denaturing conditions.
- The agarose NTA has high stability to CIP (cleaning in place) up to 1.0M NaOH when not Ni loaded.
- Regulatory Support File (RSF) documentation is available for Seplife® 6AG NTA/Ni/90.

Seplife® 6AG NTA/Ni/90 is a metal affinity chromatography resin based on highly cross-linked (6%) agarose functionalized with NTA and loaded with nickel and has a particle size in the range 45-165 micron.

2. Properties

Product	Seplife® 6AG NTA/Ni/90
Appearance	Light blue spherical beads
Type	Ni Metal affinity chromatography resin (NTA)
Matrix	6% cross-linked agarose
Ligand	Ni/Nitrilotriacetic acid
10% Dynamic binding capacity (mg /ml)**	≥40
Particle size range (μm)	45-165
pH stability	3-12 (operational), 2-14 (CIP)
Chemical Stability	Avoid using Chelating agents including citrate derivatives
Flow rate* (cm/h)	Max 750cm/h
Shipped as	20% ethanol slurry

*Testing conditions: Chromatography column 16mm×200mm; column bed height 20cm; temperature 25° C; mobile phase water.

** Testing conditions: Binding buffer: PBS,15mM imidazole，pH 7.3 Elution buffer：PBS, 250mM imidazole, pH 7.3 Sample : His-tag protein Column 8mm*100mm, room temperature, Retention time 2 minutes.

3. Instructions

3.1 Column packing

Column loading should be performed in accordance with standard operating procedures. It is important to ensure that each material is at its working temperature, and and when possible, the chromatography media may be degassed before column packing.

3.2 Equilibration

Equilibrate the column with an appropriate 2-5 column volume buffer. Ensure the conductivity and pH of the effluent are exactly the same as the buffer.

3.3 Sample feeding

1. The sample is generally dissolved in the equilibration buffer solution with pH 6-8, and increasing the pH of the loading buffer can increase the loading capacity.

2. The buffer should not contain metal chelating agents such as EDTA and citrate, and it is best to avoid reducing agents such as mercaptoethanol and DTT.

3. Commonly used buffers include 10-100 mmol/L sodium phosphate, 20-200 mmol/L Tris-HCl, etc.

4. Generally, 0.15-0.5mol/L NaCl should be added to the buffer to eliminate any ion exchange interactions.

5. When using the nickel chelate agarose media for the first time, it is recommended to use 50mmol/L PBS (50 mmol/L NaH_2PO_4, 0.5 mol/L NaCl, pH 7.4) as the initial buffer.

3.4 Elution

Typically, the following methods can be used for protein elution:

1. Lower pH: most proteins will be eluted at pH 6-4 (also at pH 3-4), the buffer can be sodium acetate, and phosphate buffer systems.

2. Competitive elution: linearly increase or one-step increase the concentration of competing substances with affinity to metal ions (such as 0-1.0 mol/L imidazole, 0-50 mmol/L histidine, 0-2 mol/L NH_4Cl).

3. Chelating agents: Chelating agents such as EDTA and EGTA can bind to metal ions and release the proteins. However, this method cannot separate different proteins and will remove the metal from the resin.

Note:

1. When using for the first time, if the concentration required for elution is not certain, it is recommended to add 10mmol/L, 20mmol/L, 50mmol/L, 100mmol/L, 200mmol/L, 500mmol/L imidazole to the initial buffer solution, from low to high concentration, to elute and collect the recombinant proteins respectively, and then identify the elution results by SDS-PAGE electrophoresis and other methods. If conditions permit,

linear imidazole gradient elution can be performed to determine better elution conditions.

2. Imidazole is alkaline, and the pH needs to be adjusted using HCl after the corresponding buffer solution is prepared.

3. Elution by lowering pH and with chelating agent will cause the metal ions to fall off, and the metal ions need to be re-chelated before the next use.

For all the above elution methods, 150-500mmol/L NaCl should be added to the buffer to eliminate any ion exchange interactions.

3.5 Regeneration

1. After multiple uses or when the chelated metal ions need to be replaced, the metal must be stripped of the chromatographic media and the resin regenerated.

Metal removal method: First rinse the column with 5-10 CV of distilled water, then rinse the column with 5-10 CV of 100mmol/L EDTA, and finally use 2-3 CV of 0.5mol/L NaCl to wash off residual EDTA.

2. Columns that have been used many times generally require cleaning after metal removal.

Cleaning method: reverse wash the column with 0.1-1.0 mol/L NaOH, and keep it at 50 cm/h for 1-2 hours, which should remove the strongly bound impurities.

3. Metal ions need to be re-chelated after washing.

Chelating method: First, use 2-5 CV of distilled water to fully balance the column after previous metal removal, then use 0.1-0.3mol/L metal salt solution (chloride or sulfate soluble metal salts are suitable) to pass through the column for 5-10 CV to chelate the fresh metal ions, and finally use 5-10 CV of distilled water to remove unbound metal ions.

3.6 Cleaning-In-Place (CIP)

When it is found that the performance of the chromatographic media decreases or the back pressure increases, then a cleaning process should be applied. Before cleaning, follow the recommended operation steps to remove Ni^{2+} ions.

The chromatography media after nickel removal is generally cleaned with a reversed-phase flow rate, and the following methods can be used for cleaning:

1. Removal of proteins adsorbed by ion exchange: first use 2 mol/L NaCl solution to backwash 2 to 3 CV, then rinse with 3 to 5 CV with distilled water.

2. Removal of precipitated proteins, hydrophobic proteins and lipids: first use 1.0 mol/L NaOH to backwash

the column (50 cm/h, contact time 1-2 h), contact for more than 12 hours to remove endotoxin. Then rinse the chromatography column with 10 CV of equilibration buffer, and finally rinse the chromatography column with 5 CV of distilled water.

3. Removal of hydrophobic proteins and lipids: first wash the chromatography column with 5-10 CV of 30% isopropanol in reverse phase (contact time 15-20min), then rinse the chromatography with 10 CV of distilled water column.

4. Storage

Sealed and stored at 4~30°C (preservation solution is 20% ethanol) in a ventilated, dry and clean place, do not freeze.

5. Transportation

Avoid sunlight, rain, and heavy pressure during transportation. It is strictly forbidden to transport with toxic and hazardous materials.

6. Precautions

6.1 The sample and chromatography media must be thoroughly equilibrated with equilibration buffer before column chromatography can be performed.

6.2 The loaded column bed must have a flat surface, with no channel flow or air bubbles, otherwise it should be reloaded.

6.3 During operation, ensure that the temperature of the column and the buffer are consistent to avoid air bubbles in the column bed, which affects the purification performance.

6.4 During the elution process, the flow rate should be strictly controlled.

6.5 During sample loading and the entire elution process, prevent the column surface from drying out.

7. Ordering information

Product Name	References	Pack Size
Seplife® 6AG NTA/Ni/90	A4023202	25ml
	A4023203	100ml
	A4023204	500ml
	A4023205	1L
	A4023206	5L
	A4023207	10L

Production date: See label

Expiry Date: 5 years, under proper storage conditions

Manufacturer: Sunresin New Materials Co. Ltd.
Add:No. 135, Jinye Rd, Xi'an Hi-tech Industrial Development Zone, Shaanxi, 710076, China
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E-mail: info.lifescience@sunresin.com