ProteinIso® Ni-NTA Resin

Cat. No. DP101

Storage: at 2-8°C (20% ethanol) for two years

Description

ProteinIso® Ni-NTA Resin allows rapid affinity purification of His-tagged proteins. The His-tagged proteins bind to Ni^{2+} cations, which are immobilized on the Ni-NTA resin by 4 metal-chelating sites. After wash, the target proteins are recovered by gradient elution. The resin can be used for both native and denatured protein purification.

Resin Specifications

Resin	Cross-linked 6% agarose
Ligand	NTA
Shape	sphere
Pore size	45~165 μm
Binding capacity	10~20 mg/ml wet gel
Recommended flow rate	<300 cm/h
Highest resistance of atmospheric pressure	0.3 Mpa
pH stability	3-13

Procedures

1. Prepare Ni-NTA purification column

(1) Thoroughly resuspend the Ni-NTA resin to achieve a homogeneous suspension of the resin in the 20% ethanol storage buffer.

(2) Immediately transfer the resin into a purification column. Ensure that the bottom of the column is plugged with a stopper. Close the valve of the column. Allow the resin to settle.

(3) Equilibrate the column with 5~10 bed volume of equilibration buffer.

2. Prepare samples

To avoid blocking column, samples should be centrifuged and filtrated with 0.45 μm filter before loading.

3. Load samples and wash

Load samples and wash with 5~10 bed volume of equilibration buffer and collect the flow-through in one tube.

4. Elute

Elute target proteins with imidazole or low pH buffer.

5. Regeneration of Ni-NTA resin

(1) Wash the column/resin with 2 bed volume of 6 M GuHCl, 0.2 M acetic acid

(2) 5 bed volume of deionized water

(3) 3 bed volume of 2% SDS

(4) 1 bed volume of 25% ethanol

(5) 1 bed volume of 50% ethanol

(6) 1 bed volume of 75% ethanol

(7) 5 bed volume of 100% ethanol

(8) 1 bed volume of 75% ethanol

(9) 1 bed volume of 50% ethanol

(10) 1 bed volume of 25% ethanol

(11) 1 bed volume of deionized water

(12) 5 bed volume of 100 mM EDTA, pH 8.0

(13) 10 bed volume of deionized water

(14) 5 bed volume of 100 mM $NiSO_4$

(15) Store column/resin in 20% ethanol.

Notes

- Samples should be centrifuged and filtrated with 0.45 μm filter before loading.
- Equilibration Buffer for soluble protein
  300 mM NaCl, 50 mM sodium phosphate buffer, 10 mM imidazole, 10 mM Tris-Cl pH 8.0
- Equilibration Buffer for inclusion body
  6 M GuHCl, 100 mM sodium phosphate buffer, 10 mM Tris-HCl pH 8.0; or 8 M urea, 100 mM sodium phosphate buffer, 10 mM Tris-HCl pH8.0