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His•Bind Agarose Resin (Ni-IDA)

Product Name	Qty	Cat. No.	Remarks
His•Bind Agarose Resin (Ni-IDA)	10 ml	EBE-1031	pre-charged with Ni ion, 75% slurry

Description

Elpis Biotech's His•Bind agarose resin is designed for rapid purification of recombinant proteins containing His•Tag sequence by IMAC. The His•Tag sequence binds to Ni^{2+} cations, which are immobilized on the His•Bind agarose resin. After unbound proteins are washed away, the target protein is recovered by elution with imidazole containing wash buffer. The His•Bind agarose resin can be regenerated and reused several times.

Features

- **Matrix** Agarose CL4B
- **Activation** Epoxy
- **Ligand** Iminodiacetic acid (Ni ion pre-charged)
- **Linker Size** 12-atom space linker
- **Binding Capacity** 5-10 mg polyhistidine tagged proteins/ml resin
- **Bead Size** 45-165 μm
- **pH Stability** pH 2 to 14
- **Storage** 75% suspension in 20% ethanol

Storage Condition

Store at 4℃, do not freeze

Reagent Compatibility

β-Mercaptoethanol	< 10 mM
CHAPS	< 1%
Ethanol	30%
Ethylene glycol	30%
HEPES	50 mM
Glycerol	20%
Guanidine HCl	6 M
Imidazole	500 mM at pH 7.0–8.0, for elution
KCl	500 mM
MES	20 mM
MOPS	50 mM
NaCl	1.0 M
NP-40	1%
SDS	< 1%
TRIS	50 mM
Triton-X 100	<1%
Urea	8 M

• Reagent compatibility shows the maximum concentrations of each reagent tested. Higher levels may be acceptable, but they should be tested before use. Note that some of these reagents may partially or completely denature your protein. EDTA or EGTA can not be used completely.

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Protocol

Resin preparation

1. Prepare 13 volumes 1x Binding buffer, 6 volumes 1x Washing buffer, and 6 volumes 1x Elution buffer before (see below for buffer composition).
2. Gently mix the bottle of His•Bind agarose resin by inversion until completely suspended. Using a wide-mouth pipette, transfer the desired amount of agarose resin slurry to a column. Allow the resin to pack under gravity flow.
3. When the level of storage buffer drops to the top of the column bed, use the following sequence of washes to equilibrate the column:
   3 volumes of sterile deionized water

   3 volumes 1x binding buffer

** Caution : The resins are supplied as pre-charged form with Ni ion.

Column chromatography

1. Allow the Binding buffer to drain to the top of the column bed and load the column with the prepared extract. A flow rate of about 10 column volumes per hour is optimal for efficient purification. If the flow rate is too fast, more impurities will contaminate the eluted fraction.
2. Wash the column with 10 volumes of 1x Binding buffer.
3. Wash the column with 6 volumes of 1x Wash buffer.
4. Elute the bound protein with 6 volumes of 1x Elute buffer. Alternatively, 6 volumes of Strip buffer may also be used to remove the protein by stripping the Ni^{2+} from the column. The eluate may be captured in fractions (e.g. 1 ml fractions) if desired.

Buffers Used

1. Buffers for purification under native conditions

1X Bind buffer (use for lysis and binding): 0.5 M NaCl, 5 mM imidazole, 20 mM Tris-HCl, pH 7.9

1X Wash buffer: 0.5 M NaCl, 60 mM imidazole, 20 mM Tris-HCl, pH 7.9

1X Elute buffer: 0.25 M NaCl, 0.5 M imidazole, 10 mM Tris-HCl, pH 7.9

1x Striping buffer: 0.5 M NaCl, 100 mM EDTA, 20 mM Tris-HCl, pH 7.9

1x Charging buffer: 50 mM $NiSO_4$

2. Buffers for purification under denaturing conditions

Denaturing Lysis/Bind buffer: 6 M Guanidine-HCl, 0.1 M NaH_2PO_4, 0.01 M Tris-Cl, pH 8.0

(optionally 8 M urea, 0.1 M NaH_2PO_4, 0.01 M Tris-Cl, pH 8.0)

Denaturing Wash buffer: 8 M urea, 0.1 M NaH_2PO_4, 0.01 M Tris-Cl, pH 6.3

Denaturing Elution buffers: 8 M urea, 0.1 M NaH_2PO_4, 0.01 M Tris-Cl, pH 5.9

(optionally 8 M urea, 0.1 M NaH_2PO_4, 0.01 M Tris-Cl, pH 4.5)