For research use only

ISO9001

# His•Bind Magnetic Agarose Resin (Ni-IDA)

| Product Name | Qty | Cat. No. | Remarks |
|---|---|---|---|
| **His•Bind Magnetic Agarose Resin (Ni-IDA)** | 2 ml | EBE-1032 | 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. Para-magnetic particles are embedded onto agarose bead enables to purify in a batch style high-throughput purification using a magnet.

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 4B, with magnetite, cross-linked
- **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** 10-25 μm
- **pH Stability** pH 2 to 14
- **Storage** 75% suspension in PBS with 20% ethanol

## Storage

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.

Technical Services:
e-mail : elpis@elpisbio.com
www.elpisbio.com

㈜ 엘피스바이오텍
ELPIS BIOTECH

## Protocol

### Resin preparation

1. Prepare 1x Binding buffer, 1x Washing buffer, and 1x Elution buffer before purification (see below for buffer composition).
2. Gently mix the tube of His•Bind magnetic agarose resin by inversion until completely suspended. Using a wide-mouth pipette, transfer the desired amount of agarose resin slurry to a column.
3. Wash the resin with 3 volumes 1x binding buffer.

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

### Small Scale Purification

1. Resuspend the cells in 1X Bind buffer at 2–5 ml per gram wet weight. The amount of cells required depends on the expression level of the His•Tag fusion protein and the expression system used. The binding capacity of His•Bind magnetic agarose resins is protein-dependent and normally lies between 5–10 mg/ml. Lysis/binding buffer contains 5 mM imidazole to minimize binding of untagged, contaminating proteins and increase purity with fewer wash steps. If the His•Tag fusion protein does not bind under these conditions, the amount of imidazole should be reduced to 1–5 mM. With His•Tag fusion proteins exhibiting high binding affinities, the imidazole concentration can be increased to 20 mM.
2. Add lysozyme to 1 mg/ml and incubate on ice for 30 min
3. Sonicate on ice. Use six 10 sec bursts at 200–300 W with a 10 sec cooling period between each burst. Use a sonicator equipped with a microtip
4. (Optional) If the lysate is very viscous, add RNase A (10 μg/ml) and DNase I (5 μg/ml) and incubate on ice for 10–15 min.
5. Centrifuge lysate at 13,000rpm for 20–30 min at 4˚C to pellet the cellular debris. Save supernatant and store –20˚C.
6. Add 100-500 μl of cleared lysate to the 20 μl His•Bind magnetic agarose resin and mix gently by shaking (200rpm on a rotary shaker) at 4˚C for 60 min.
7. Centrifuge (optionally by magnetic separator) lysate- His•Bind magnetic agarose mixture at 3,000rpm for 30 sec at 4 ˚C.
8. Remove supernatants. Save flow-through for SDS-PAGE analysis.
9. Wash twice with 500 μl 1x Wash buffer by repeat of mixing and centrifugation at 3,000rpm for 30 sec at 4 ˚C, collect wash fractions. Save wash fractions for SDS-PAGE analysis.
10. Elute the protein with 4 × 20 μl 1x Elution buffer. Collect the eluate in four tubes and analyze fractions by SDS-PAGE. Alternatively, 10 -100 μl of Strip buffer may also be used to remove the protein by stripping the $Ni^{2+}$ from the column.

### 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 $NaH_2PO_4$, 0.01M 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)