FOR RESEARCH USE ONLY!

Hi-Bind™ Ni QR Agarose Beads

rev. 9/13

Store at 4°C. Do not freeze.

Cat. No.	
6562-1	**Hi-Bind™ Ni QR Agarose Beads,** 1 ml settled resin
6562-10	**Hi-Bind™ Ni QR Agarose Beads,** 10 ml settled resin
6562-100	**Hi-Bind™ Ni QR Agarose Beads,** 100 ml settled resin
6562-500	**Hi-Bind™ Ni QR Agarose Beads,** 500 ml settled resin

Binding Capacity: Up to 60 mg target protein/ml of settled resin. The binding capacity for individual proteins may vary.
Content: Supplied as 50% slurry (e.g., 1 ml of settled resin is equivalent to 2 ml of 50% slurry) in 20% ethanol.
Flow Rate: Higher than 4 cm/min.

Description:
Basic research of the physico-chemical properties as well as the activity of proteins is impossible without their isolation and purification. Majority of protein studies are carried out on tagged recombinant proteins expressed in various host organisms. Over 50% of these are expressed as fusions with poly-histidine purification tags. The small size and the mild conditions utilized during purification as well as the low cost of purification made this type of fusion the most popular (and in many cases, the first tag of choice). BioVision's Hi-Bind™ Ni QR Agarose Beads combines the capacity of immobilized nickel ions with the extreme flow rates of Quick Run (QR) Agarose beads. These beads deliver as much as 25% higher capacity than Ni-NTA adsorbents while dramatically decreasing purification times due to the ability to perform washing steps at linear flow rates higher than 4 cm/min.

Applications:
- Purification of native and recombinant proteins and peptides that have an affinity for metal ions.
- For purification under native or denaturing conditions
- Use for batch/gravity flow and automated FPLC purification

User Supplied Reagents and Equipments:
- Disposable batch/gravity flow column
- Buffers - Native conditions. For convenience, BioVision provides Ready-to-use Ni QR Agarose Bead Buffer Kit (Cat. # K6563-3) containing Ni QR Agarose Loading & Elution Buffer & EZLys™ Mammalian-Bacterial Protein Extraction Reagent. The following information is provided if you want to prepare your own buffers.
  1. Loading Buffer**:** 50 mM sodium phosphate, 0.3 M NaCl; pH 7.2
  2. Washing Buffer: 50 mM sodium phosphate, 0.3 M NaCl, 5-40 mM imidazole; pH 7.2
  3. Elution Buffer**:** 50 mM sodium phosphate, 0.3 M NaCl, 300 mM imidazole; pH 7.2
- Buffers - Denaturing conditions
  1. Loading Buffer**:** 50 mM sodium phosphate, 0.3 M NaCl, 6 M Guanidinium.HCl; pH 7.2
  2. Washing Buffer: 50 mM sodium phosphate, 0.3 M NaCl, 6 M Guanidinium.HCl, 5-40 mM imidazole; pH 7.2
  3. Elution Buffer**:** 50 mM sodium phosphate, 0.3 M NaCl, 6 M Guanidinium.HCl, 300 mM imidazole; pH 7.2

  Note: For FPLC & other automated applications, filter the buffers through 0.45 μm filter & degas them before use.

Purification procedure:
Equilibrate the Hi-Bind™ Ni QR Agarose Beads to room temperature (RT) if the purification is to be carried out at RT. The following protocol is for 1 ml packed Hi-Bind™ Ni QR adsorbent column or equivalent.

A. Sample Preparation: Use 0.2 to 0.5 g cell pellet per ml of settled Hi-Bind™ Ni QR beads. Homogenize or sonicate the cells in 4 to 10 volume of Loading Buffer. Centrifuge for 15 min. at 10,000 x g at 4°C and collect the supernatant. Alternatively, to obtain high yield isolation under mild conditions, use BioVision's EZLys™ Mammalian-Bacterial Protein Extraction Reagent (Cat. # K6563-3-3) & follow the Ready-to-Use Ni QR Agarose Beads Buffer Kit (Cat. # K6563-3) protocol for sample preparation.

B. Batch/Gravity - Flow Column Purification:

1. Pack the column while preparing cell lysate. Drain excess liquid from the column. Wash the beads with 2 x 5 ml of Milli Q water followed by 1 x 5 ml of Loading Buffer.
2. Load extract obtained from 0.2-0.5 g pellet to settled Hi-Bind™ Ni QR beads. Put bottom and top stoppers on the column & allow target protein to bind by slowly inverting the column for 10 min. at 4°C or by mixing the sample with the beads once every min. while keeping the column on ice in between mixes. Let the beads settle, open the top stopper, put a collection tube under the column, and remove the bottom stopper. Collect non-adsorbed material followed by 5 ml wash with Loading Buffer. Collect all following fractions in separate tubes.
3. Wash the column with 2 x 5 ml of Washing Buffer.
4. Elute the target protein with 5 x 1 ml fractions of Elution Buffer. Analyze protein content in all fractions by Bradford or BCA (Cat. #s K812, K813, K814, K818, K819) using appropriate blanks and protein standards. Determine yield and purity of target protein by electrophoresis or other analytical techniques. If necessary, repeat the purification with optimized imidazole concentration and volumes of the Washing Buffer

C. FPLC/Akta Column Purification:

1. Pack 1 ml of beads into an appropriate FPLC/Akta column. Drain excess liquid from the column. Wash the column with 15 ml of Loading Buffer.

2. Load extract obtained from 0.2-0.5 g pellet at 0.5 cm/min. linear flow rate while collecting 1 to 2 ml fractions during the whole run.
3. Wash the column with 10 ml of Wash Buffer.
4. Elute the target protein with 10 x 1 ml fractions of Elution Buffer. Analyze protein content in all fractions by Bradford or BCA (Cat. #s K812, K813, K814, K818 & K819). Determine yield and purity of target protein by electrophoresis or other analytical techniques.

D. Regeneration & Storage:

1. In case you would like to re-use the resin for purification of the same protein at a later time, we recommend that you wash the resin with 10 resin volumes of 20 mM MES or 20 mM sodium acetate pH 5 containing 0.3 M sodium chloride. Extended wash (20 or more resin volumes) with Loading Buffer will also allow its re-use.
2. For extended storage after use, wash the resin with five resin volumes of Milli Q and store as 20% ethanol suspension at 4°C. If packed in FPLC column, wash with 5 column volumes of Milli Q water followed by 5 column volumes of 20% ethanol, close input and output of the column and store at 4°C.

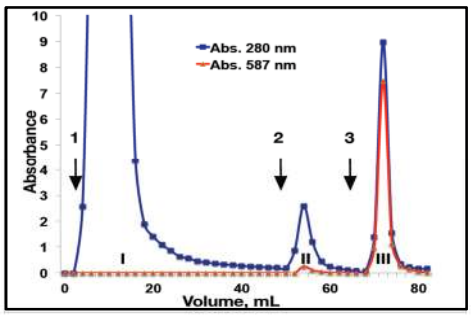

Figure 1. Purification of 6xHis-mCherry on Hi-Bind™ Ni QR Agarose Beads: 1 g of *E. coli* pellet expressing 6xHis-mCherry was extracted with 10 ml of EZLys™ Mammalian-Bacterial Protein Extraction Reagent. 8 ml was loaded at 0.5 ml/min. on 1 cm.i.d x 1.8 cm length column equilibrated with Loading Buffer. The column was washed at 1 ml/min. with Loading Buffer (Arrow 1, Peak I), 25 mM imidazole in the Loading Buffer (Arrow 2, Peak II) and 6xHis-mCherry was eluted with 250 mM imidazole in the Loading Buffer (Arrow 3, Peak III).

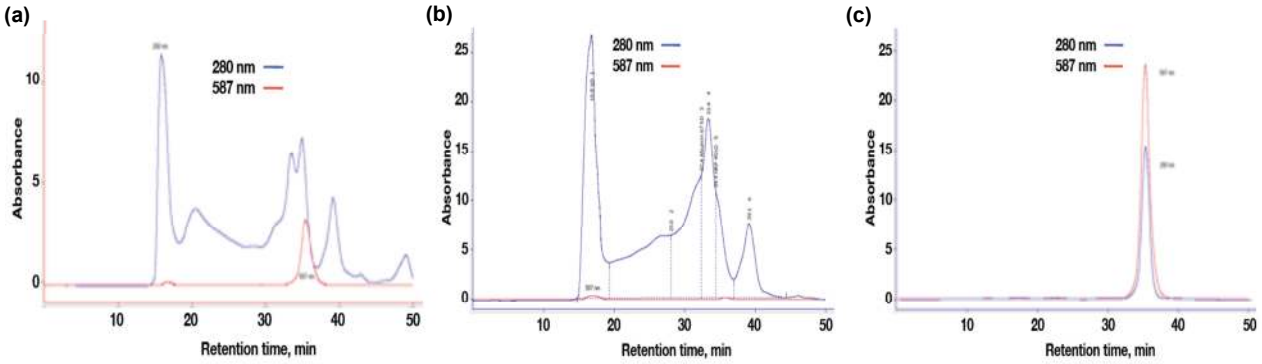

Figure 2 a. Analytical SEC of *E. coli* extract loaded on Hi Bind™ Ni QR Agarose Beads column: 200 µl of 10:1 dilution of the extract was loaded and analyzed at 0.5 ml/min. on Superdex 200 HR 10/30 column in 50 mM sodium phosphate, 0.2 M NaCl; pH 7.5. **b. Analytical SEC of Peak I, Figure 1:** 200 µl of pooled fractions of the non-adsorbed material were loaded and analyzed at 0.5 ml/min. on Superdex 200 HR 10/30 column in 50 mM sodium phosphate, 0.2 M NaCl; pH 7.5. Specific 587 nm absorbance peak of mCherry at approximately 36 min. is almost completely depleted. **c. Analytical SEC of Peak III, Figure 1:** 200 µl of pooled fractions of eluted 6xHis-mCherry were desalted on PD-10 column, loaded and analyzed at 0.5 ml/min. on Superdex 200 HR 10/30 column in 50 mM sodium phosphate, 0.2 M NaCl; pH 7.5. Specific 587 nm absorbance peak of mCherry at approximately 36 min. overlaps with the single 280 nm absorbance peak.

RELATED PRODUCTS:

Heparin Sepharose (6553)
Glutathione Sepharose (6555)
Ready-to-use Ni QR Agarose Beads Buffer Kit (K6563-3)
Protein A-Agarose (6526)
Protein A-Sepharose Column (6508)
Protein G-Sepharose (6511)
Protein L-Sepharose (6531)
Protein A/G-Sepharose (6503)
Protein A/G/L-Sepharose (6541)
Heparin Sepharose Column (6554)
Jacalin Sepharose (6561)
Hi-Bind™ Protein A-Agarose (6520)
Protein A-Sepharose (6501)
Hi-Bind™ Protein G-Agarose (6513)
Protein G-Sepharose Column (6518)
Protein L-Sepharose Column (6538)
Protein A/G-Sepharose Column (6528)
Protein A/G/L-Sepharose Column (6548)

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