FOR RESEARCH USE ONLY!

Ready-to-Use Ni QR Agarose Beads Buffer Kit

rev. 1/14

(Store at 4°C)

Cat. No.

K6563-3 Ready-to-Use Ni QR Agarose Beads Buffer Kit, contains Ni QR Agarose Loading Buffer, Elution Buffer & Extraction Reagent

I. Introduction:

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 Ready-to-Use Ni QR Agarose Beads Buffer Kit works seamlessly with Hi-Bind™ Ni QR Agarose Beads (Cat. # 6562) or equivalent from the isolation of target to obtaining highly purified and biologically active protein. These Buffers & Extraction Reagent deliver efficient extraction of target protein (as high as 96% efficiency of SDS boiling method) while preserving its integrity. The buffer components are designed to minimize the metal ion leaching while preserving the biological activity of precious target proteins. This kit is sufficient for purification of up to 300 mg of target protein using 5 ml of Hi-Bind™ Ni QR Agarose Beads (Cat. # 6562) or equivalent under batch/gravity flow conditions.

II. Applications:

- Purification of native and recombinant proteins and peptides that have an affinity for metal ions
- For purifications under native conditions

III. Kit Contents:

Components	K6563-3	Cap Code	Part Number
EZLys™ Mammalian-Bacterial Protein Extraction Reagent	20 ml	WM	K6563-3-1
Ni QR Agarose Loading Buffer	120 ml	NM	K6563-3-2
Ni QR Agarose Elution Buffer (0.3 M imidazole)	40 ml	NM	K6563-3-3

IV. Storage and Handling:

Store at 4°C. Read the entire protocol before performing the experiment.

V. User Supplied Reagents or Equipments:

- Hi-Bind™ Ni QR Agarose beads (Cat. # 6562) or equivalent, Benzonase, E. Coli Recombinant (Cat. # 7680), gravity-flow column(s)

VI. 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 (Cat. # 6562) or equivalent.

A. Sample Preparation: Add 4 ml EZLys™ Mammalian-Bacterial Protein Extraction Reagent to 0.2 to 0.5 g cell pellet. Pipet up and down to fully re-suspend the pellet & add 1 µl of Benzonase. Mix gently by pipetting up and down. Incubate with gentle shaking for 10 min. at room temperature*. Aliquot to four (4) cold 1.5 ml centrifuge tubes & centrifuge at 10,000 – 12,000 x g for 20 min. at 4°C. Transfer the supernatant to a cold 14 ml falcon tube and keep on ice (we recommend to purify protein immediately after preparing cell lysate).

*** Note**: At the end of this incubation period, there should be no visible particles. If any cell pellet fragments are present, then resuspend them by pipetting up and down and incubate for an additional 1-2 min.

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 (1:10 dilution of Elution Buffer with Loading 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.

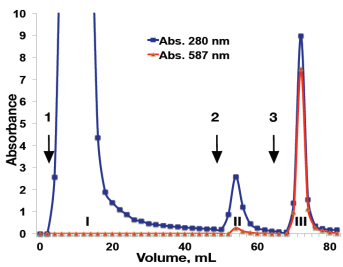

Figure 1. Purification of 6xHis-mCherry using Ready-to-Use Ni QR Agarose Beads Buffer Kit: 1 g of *E. coli* pellet expressing 6xHis-mCherry was extracted with 10 ml of EZLys™ Mammalian-Bacterial Protein Extraction Reagent. 8 ml was loaded at 0.5 ml/min. on 1 cm.i.d x 1.8 cm length column equilibrated 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).

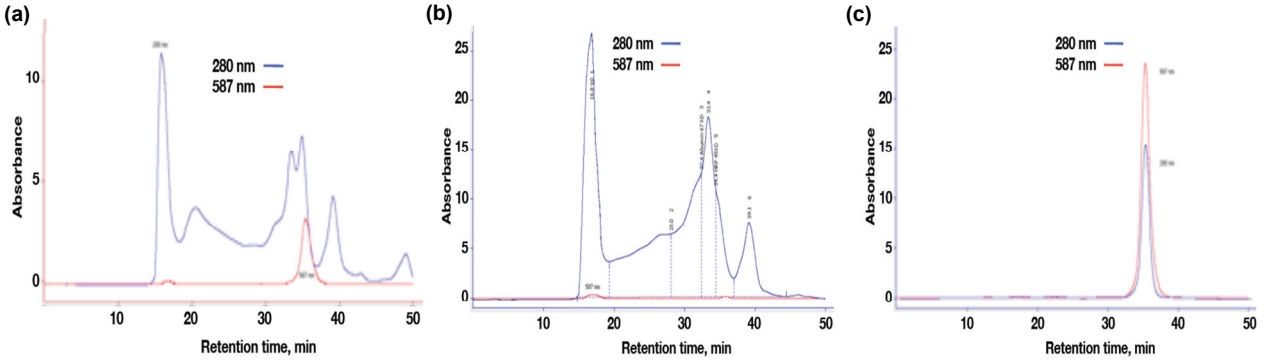

Figure 2 a. Analytical SEC of *E. coli* extract loaded on Hi Bind™ Ni QR Agarose Beads column: 200 µl of 10:1 dilution of the extract was loaded and analyzed at 0.5 ml/min. on Superdex 200 HR 10/30 column in 50 mM sodium phosphate, 0.2 M NaCl; pH 7.5. **b. Analytical SEC of Peak I, Figure 1:** 200 µl 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.

VII. RELATED PRODUCTS:

Hi-Bind™ Ni QR Agarose Beads (K6562)
Heparin Sepharose Column (6554)
Jacalin Sepharose (6561)
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)
Benzonase, E. Coli Recombinant (7680)
Heparin Sepharose (6553)
Glutathione Sepharose (6555)
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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