FOR RESEARCH USE ONLY!

Ni-IDA Spin Columns

12/13

Store at 4°C. Do not freeze.

Cat. No.: 6567-25 **25 Ni-IDA Spin Columns**

Salient Features:

Binding Capacity:	Upto 2.8 mg poly-histidine tagged proteins per column.
Content:	25 Ni-IDA spin columns, each containing 100 µl high performance Hi-Bind™ Ni QR Agarose Beads (Cat. #6562).
Bead content:	A 50% slurry (e.g., 1 ml of settled resin is equivalent to 2 ml of 50% slurry) in 20% ethanol.

Recommended comprehensive product: K6567-25, Ready-to-Use Ni-IDA Spin Purification Kit – A comprehensive kit with the Ni-IDA Spin Columns, column loading buffer, wash buffer, elution buffer and centrifuge tubes.

Description:

Protein activity and functional studies rely largely on the availability of purified target proteins. Majority of protein studies are carried out on tagged recombinant proteins expressed in various host organisms. Over 50% of these tagged recombinant proteins are expressed as fusions with poly-histidine purification tags. The small size and the mild conditions utilized during His-tagged protein purification as well as the associated low costs makes this type of fusion the most popular (and in many cases, the first tag of choice). BioVision's Ni-IDA Spin Columns are ideal for small-scale purification of these poly-histidine-tagged proteins rapidly. Each of these columns is filled with 100 µL of high performance Hi-Bind™ Ni QR Agarose Beads (Cat. #6562), enabling efficient capture and purification of up to 2.8 mg of poly-histidine tagged proteins per column. The small volume of beads inside the columns is optimal for loading various amounts of sample. At normal expression levels, it permits efficient displacement of non-tagged protein impurities by the more strongly binding poly-histidine tagged targets and improves the purity of the final product. The yield and purity of purification are protein specific and depends on various factors like the expression level, structure, solubility etc. The beads packed in each column deliver as much as 25% higher capacity than Ni-NTA adsorbents while dramatically decreasing purification times due to the ability to perform centrifugation.

Applications:

- Ideal for small scale, one – step purification of poly-histidine tagged recombinant proteins under native conditions.
- Ideal for small scale, one – step purification of native proteins and peptides that have an affinity for metal ions.
- Screening of expression levels in a HTP manner.
- Applicable for purification under native and denaturing conditions.

User Supplied Reagents and Equipment:

Buffer for native purification: For convenience, BioVision Ready-to-use Ni QR Agarose Bead Buffer Kit (Cat. # K6563-3) provides a complete set of buffers needed for this application. The kit contains EZLys™ Mammalian-Bacterial Protein Extraction Reagents, Ni QR Agarose Loading buffer, Elution Buffer. Washing buffer is to be prepared by diluting Elution Buffer in Loading buffer (1:10).

Recommended buffer compositions for native purification:

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

Recommended buffer compositions for denaturing purification

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

Microcentrifugation tubes: regular 1.5 ml and 2.0 ml microcentrifugation tubes

Centrifuge with a fixed angle or a swing bucket rotor for 1.5 ml or 2.0 ml microcentrifuge tubes.

Spectrophotometer/electrophoresis/ analytical apparatus to determine the final protein yield and purity.

Purification Procedure

A. Sample / Lysate preparation

1. Lyse the cell pellet (up to 150 mg) in 600 µL Loading Buffer (4 volume of cell pellet, V/W), by any preferred technique like homogenization, sonication, freeze-thaw, etc (We recommend adding protease inhibitors such as PMSF, AEBSF etc., in Loading Buffer to help minimize proteolysis). Alternatively, cell pellets can be lysed by resuspension in BioVision's EZLys™ Mammalian-Bacterial Protein Extraction Reagent (K6563-3-1), followed by incubation with Benzonase nuclease (Cat. #7680).
2. Centrifuge for 15 min. at 10,000 x g at 4°C and collect the supernatant / clear cell lysate.

B. Column preparation

1. Snap off the bottom closure **(save it)** (the distal end of the closure works to plug column during step B5) and slightly loosen the top cap.
2. Place the column in a 2.0 ml microcentrifuge tube, centrifuge at 150 g x 1 min and discard the storage solution in the tube.
3. Place the column back into the centrifuge tube and add 500 µl ddH_2O to column. Centrifuge at 150 g x 1 min and discard the solution in tube again.

4. Place the column back in the centrifuge tube and add 500 µl of loading buffer to column. Centrifuge at 150 g x1 min and discard the solution.
5. Close the column with the bottom plug, which was preserved at step 1. The column is now ready for loading the sample.

C. Purification

1. Add 500 µl lysate to the column, close the top cap tightly and mix the column end-over-end for 10 minutes at 4˚C
2. Slightly loosen the top cap, then remove bottom plug, and place column in a 2.0 ml microcentrifuge tube and centrifuge at 150 g x 1 min (maximal loading volume is about 550 µl; Repeat Steps C1 and 2 for larger sample volumes as required).
3. Place the column back in a tube, add 500 µl Washing Buffer, centrifuge at 150 g x 1 min and discard the solution
4. Repeat step C3 for 3 more washings.
5. Transfer the column to a clean 1.5 ml tube, add 250 µl elution buffer, tap the column gently to mix the beads and solution and let it sit for 1 min
6. Spin at 150 g x 1 min to collect the first elution in the tube.
7. Repeat 5 and 6 to elute one more time.
8. Estimate the protein concentration of the eluted fractions using their absorbance at 280 nm (OD280). To remove excess imidazole, which in many cases interferes with downstream applications, use EZ-Desalt™ Spin Desalting Columns (Cat. # 6564-25) or DiaEasy™ Dialyzer tubes (Cat # K1000 – K1021). Ultrafiltration devices (10K Spin Column, Cat. #1997) can be used to concentrate your samples.

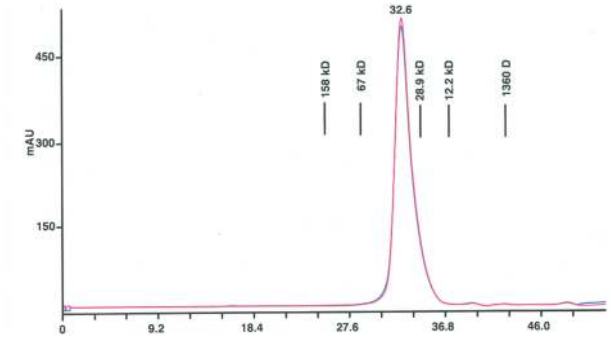

Figure 1. Analytical SEC of E. coli Extract Expressing 6xHis-mCherry Purified with Ni-IDA Spin Columns: 0.15 gms pellet extract was analyzed on Superdex 200 HR 10/30 column. Specific 587 nm absorbance (red) peak mCherry (approximately 16 mins), overlaps with single 280 nm absorbance (blue) peak, suggesting high purity levels.

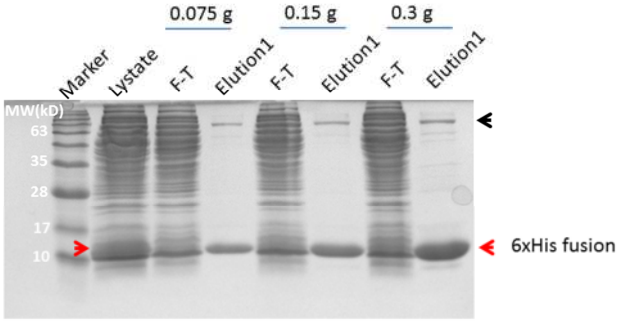

Figure 2. SDS-PAGE Image of His-tagged Proteins Purified with Ni-IDA Spin Columns: *E. coli* pellets expressing a 6xHis-tagged protein of approximate 13.3 kDa (0.075g, 0.15g and 0.3g pellet) were lysed with 4 volumes of EZLys™ Mammalian-Bacterial Protein Extraction Reagent (Cat #6563-3-1). The 6xHis fusion lysate was purified with Ni-IDA Spin Purification Kit (K6567-25). The lysate, flow-through and the first elution were resolved by SDS-PAGE, and stained with Coomassie. The red arrow indicates 6xHis fusion, and the black arrow may be the oligomeric form.

Figure 1 & 2 show examples of purification results of two different hexa-histidine tagged proteins purified using this Ni-IDA Spin Column, with yields of 2.8 mg/column and 1.4 mg/column respectively.

RELATED PRODUCTS:

Ready-to-Use Ni-IDA Spin Purification Kit (K6567-25)
Hi-Bind™ Ni QR Agarose Beads (6562)
Benzonase Nuclease (Cat. #7680)
10K Spin Column (1997)
Ready-to-use Ni QR Agarose Beads Buffer Kit (K6563-3)
Protein G-Sepharose Column (6518)
Protein A/G-Sepharose Column (6528)
DiaEasy™ Dialyzer (250 µl) MWCO 6-8 kDa (K1020)
DiaEasy™ Dialyzer (250 µl) MWCO 25 kDa (K1022)
DiaEasy™ Dialyzer (800 µl) MWCO 3.5 kDa (K1018)
DiaEasy™ Dialyzer (3 ml) MWCO 3.5 kDa (K1012)
DiaEasy™ Dialyzer (3 ml) MWCO 12-14 kDa (K1014)
DiaEasy™ Dialyzer (3 ml) MWCO 50 kDa (K1016)

Ready-to-use Ni QR Agarose Beads Buffer Kit (6563-3)
EZ-Desalt™ Spin Desalting Columns (6564-25)
Glutathione Sepharose (6555)
Protein A-Sepharose Column (6508)
Protein L-Sepharose Column (6538)
Protein A/G/L-Sepharose Column (6548)
DiaEasy™ Dialyzer (250 µl) MWCO 12-14 kDa (K1021)
DiaEasy™ Dialyzer (800 µl) MWCO 1 kDa (K1017)
DiaEasy™ Dialyzer (800 µl) MWCO 6-8 kDa (K1019)
DiaEasy™ Dialyzer (3 ml) MWCO 6-8 kDa (K1013)
DiaEasy™ Dialyzer (3 ml) MWCO 25 kDa (K1015)

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