Datasheet for ABIN3199240

Ni-NTA MagBeads

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Overview

Quantity:	1 mL
Application:	Pull-Down Assay (Pull-Down), Purification (Purif), Separation (Sep)

Product Details

Purpose:	Specific binding and purification of his-tagged proteins
Brand:	HighSpec
Specificity:	Affinity to His-tagged proteins
Characteristics:	• High binding capacity >70 mg/mL settled beads • Stable in buffer containing 10 mM DTT and 1 mM EDTA • Delivered as 25 % suspension • Average magnetic agarose bead size: 25 µm
Components:	Affinity Magnetic Agarose
Material not included:	• Lysis Buffer • Wash Buffer • Elution Buffer • Ice bath • Refrigerated microcentrifuge (min 10,000 x g) • Micropipettor and Micropipetting tips • 1.5 mL conical microcentrifuge tubes • Magnetic holder for microcentrifuge tubes (for separation of magnetic beads) • pH meter • End-over-end shaker • SDS-PAGE buffers, reagents and equipment Optional: Western Blot reagents and equipment

Product Details

Bead Ligand:	Ni-NTA
Bead Size:	25 µm

Application Details

Application Notes:	For use with: E.coli and eukaryotic cell lysates, cell culture supernatants Assay Time Procedure: 3-4 h
Comment:	KD of NTA to 6xHis-tag: ca 10 µM Sample Volume for an assay: >10 mL E.coli culture volume or corresponding quantity. Protocol can be scaled up easily.
Protocol:	Purification of his-tagged protein using magnetic beads, and pull-down assays
Reagent Preparation:	**A Purification under native conditions:** • Native Lysis buffer: NaH2PO4 50 mM, NaCl 300 mM, Imidazole 10 mM, pH 8 Optional: Add 1 tablet protease inhibitor cocktail to the Lysis Buffer. Tip: Lysis Buffer contains 10 mM imidazole to prevent binding of untagged proteins. If His-tagged proteins do not bind under these conditions, reduce the imidazole concentration to 1-5 mM. • Native Wash buffer: NaH2PO4 50 mM, NaCl 300 mM, Imidazole 20 mM, pH 8 • Native Elution buffer: NaH2PO4 50 mM, NaCl 300 mM, Imidazole 250- 500 mM, pH 8 Additional chemicals required: Lysozyme, Benzonase® nuclease, Optional: Protease inhibitor cocktail **B Purification under denaturing conditions:** • Denaturing Lysis buffer: NaH2PO4 100 mM, Tris base 10 mM, Urea 8 M, pH 8.0, Optional: Benzonase® nuclease (e.g. Merck Milipore, #707464) • Denaturing Wash buffer: NaH2PO4 100 mM, Tris base 10 mM, Urea 8 M, pH 6.3NaH2PO4 100 mM • Denaturing Elution buffer: NaH2PO4 100 mM, Tris base 10 mM, Urea 8 M, pH 4.5 Tip: If the target protein is acid-labile, elution can be performed with 250-500 mM imidazole. Note: Due to urea dissociation, adjust the pH immediately before use.
Assay Procedure:	**A Protocol** for purification under **native conditions:** 1. Thaw the E. coli cell pellet on ice. Optional: Freezing the cell pellet at -20 °C for 30 min prior to incubation at room temperature improves lysis by lysozyme.

2. Resuspend the cell pellet in 1 mL Native Lysis Buffer supplemented with 1 mg/mL lysozyme.
3. Add 6 U Benzonase® (3 units/mL bacterial culture) to the lysate to reduce viscosity caused by genomic DNA.
4. Incubate for 30 min on ice, if necessary. Otherwise, incubating at room temperature (20-25 °C) may be more efficient.
5. Centrifuge the lysate for 30 min at 10,000xg and 4 °C. Collect the supernatant. Note: The supernatant contains the cleared lysate fraction. We recommend to take aliquots of all fractions for SDS-PAGE analysis.
6. Resuspend the HighSpec Ni-NTA MagBeads by vortexing. Transfer 40 µL of the 25 % magnetic bead suspension into a conical microcentrifuge tube. Note: Depending on the protein expression rate, the quantity of magnetic bead suspension can be adjusted from 2-200 µL.
7. Add 500 µL Native Lysis Buffer and mix by vortexing. Place the tube on a magnetic microtube stand until the beads are separated and discard the supernatant.
8. Pipet 1 mL of the cleared lysate onto the equilibrated magnetic beads, and incubate the lysate-magnetic bead mixture at 4 °C for 1 h on an end-over-end shaker.
9. Place the tube on the magnetic microtube stand until the beads separate and remove the supernatant. Tip: Briefly centrifuge the sample before placing it on the magnetic separator in order to collect liquid from the lid.
10. Remove the tube from the magnet. Add 500 µL Native Wash Buffer and mix by vortexing. Place the tube again on the magnetic microtube stand and allow the beads to separate. Remove the supernatant.
11. Repeat step 10 twice.
12. Elute the His-tagged protein using 100 µL Native Elution Buffer. Note: Depending on the protein expression rate and desired protein concentration, the elution volume can be adjusted from 25 to 500 µL.
13. Repeat step 12xxx Collect each elution fraction in a separate tube and determine the protein concentration of each fraction.
14. Analyze all fractions by SDS-PAGE. Note: Do not boil membrane proteins. Instead, incubate the sample at 46 °C for 30 min in preparation for SDS-PAGE analysis.
15. Optional: Perform Western Blot experiment using PentaHis Antibody.

B Protocol for purification under **denaturing conditions:**

1. Thaw the E. coli cell pellet on ice.
2. Resuspend the cell pellet in 1 mL Denaturing Lysis Buffer. Optional: Benzonase® can be added to the lysate to reduce viscosity caused by nucleic acids (3 U/mL bacterial culture). In addition, nucleic acids can be sheared by passing the lysate 10 times through a fine-gauge needle.
3. Incubate at room temperature for 30 min on an end-over-end shaker.
4. Centrifuge the lysate for 30 min at room temperature and 10,000 x g. Collect the supernatant. Note: The supernatant contains the cleared lysate fraction. We recommend to take aliquots of all fractions for SDS-PAGE analysis.
5. Pipet 1 mL of the cleared lysate into a conical microcentrifuge tube.

Application Details

6. Resuspend the HighSpec Ni-NTA MagBeads by vortexing. Transfer 40 µL of the 25% magnetic beads suspension onto the lysate. Note: Depending on the protein expression rate and desired protein concentration, the elution volume can be adjusted from 25 to 500 µl.
7. Incubate the lysate-magnetic bead mixture at room temperature for 1 h on an end-over-end shaker.
8. Place the tube on the magnetic microtube stand until the beads separate and remove the supernatant. Tip: Briefly centrifuge the sample before placing it on the magnetic separator in order to collect liquid from the lid.
9. Remove the tube from the magnet. Add 500 µL Denaturing Wash Buffer and mix by vortexing. Place the tube again on the magnetic microtube stand and allow the beads to separate. Remove the supernatant.
10. Repeat step 9 twice.
11. Elute the His-tagged protein using 100 µL Denaturing Elution Buffer. Note: Depending on the protein expression rate and desired protein concentration, the elution volume can be adjusted from 25 to 500 µl.
12. Repeat step 11. Collect each elution fraction in a separate tube and determine the protein concentration of each fraction.
13. Analyze all fractions by SDS-PAGE. Note: Do not boil membrane proteins. Instead, incubate samples at 46˚C for 30 min in preparation for SDS-PAGE analysis.
14. Optional: Perform Western Blot experiment using PentaHis Antibody.Tip: If the target protein is acid-labile, elution can be performed with 250-500 mM imidazole.

Calculation of Results:	Analyze by SDS-PAGE, Bradford Assay or spectrophotometrically.
Assay Precision:	3 - 4 h
Restrictions:	For Research Use only

Handling

Format:	Liquid
Storage:	4 °C

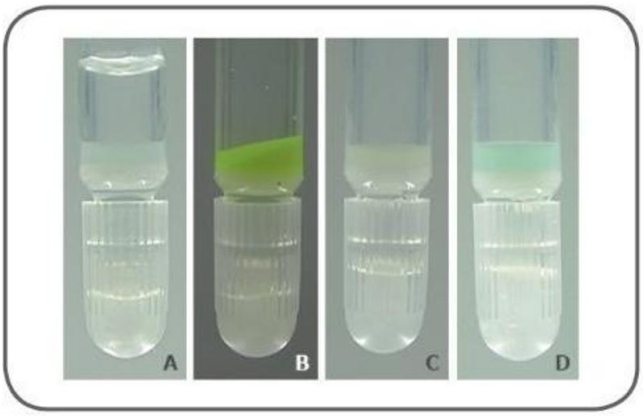
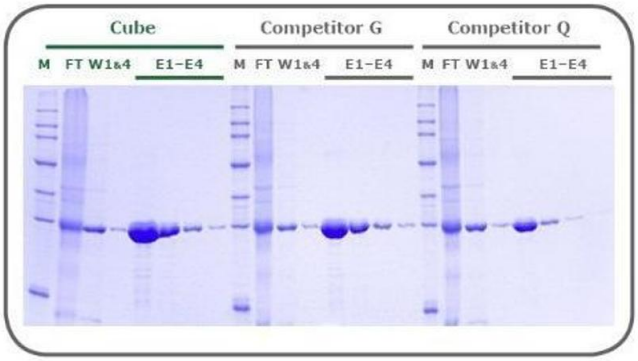


Image 1. Fig. 4: PureCube Ni-NTA Agarose is robust against oxidation and regenerable. PureCube Ni-NTA Agarose was exposed to 5mM DTT for 1 h (A). After demonstrating that it could still bind GFP (B), the resin was washed, stripped (C), and reloaded with Ni2+ (D) following standard Cube protocol (see Cube Protocols & Datasheets).



SDS-PAGE

Image 2. Fig. 3: NTA is robust in the presence of reducing and chelating agents. GFP-His was purified on gravity columns containing PureCube Ni-NTA Agarose after exposing the resin for 1 h to 3 concentrations of DTT or EDTA. NTA exhibits a shallow decay rate in binding capacity.

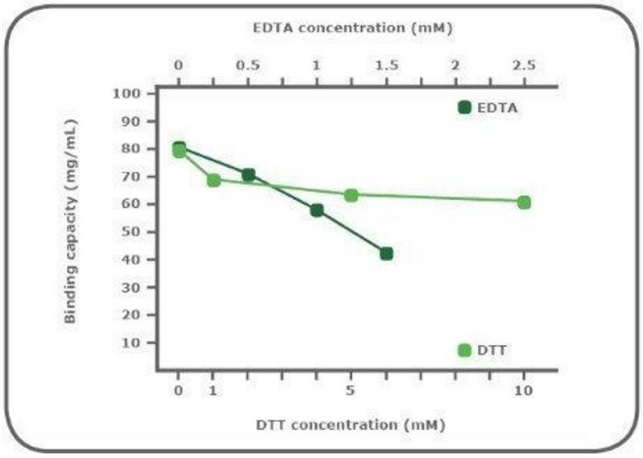


Image 3. Fig. 2: Over 20% more yield obtained with PureCube Ni-NTA Agarose. SDS-PAGE of GFP expressed in E. coli and purified in gravity columns with PureCube Ni-NTA Agarose and Ni-NTA resin from Competitor Q. 80 mg/mL protein yield was obtained with PureCube Ni-NTA Agarose (E1–E4, Cube) compared to 65 and 48 mg/mL, respectively, with the widely used alternative resins G and Q (E1–E4, Competitor G / Competitor Q).

Please check the product details page for more images. Overall 4 images are available for ABIN3199240.