Datasheet for ABIN3199239

Ni-NTA Agarose

4 Images

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Overview

Quantity:	10 mL
Application:	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 • Stable in buffer containing 10 mM DTT and 1 mM EDTA • Delivered as a 50 % suspension • Average agarose bead size: 40 µm
Components:	Affinity Agarose
Material not included:	• Lysis Buffer • Wash Buffer • Elution Buffer • Ice bath • Refrigerated centrifuge for 50 mL tube (min 10,000 x g) • 50 mL centrifuge tube • Micropipettor and Micropipetting tips • Disposable gravity flow columns with capped bottom outlet, 2 ml • 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:	40 µm

Application Details

Application Notes:	For use with: E.coli and eukaryotic cell lysates, cell culture supernatants Assay Time Procedure: 4-5 h
Comment:	KD of NTA to 6xHis-tag: ca 10 µM Sample Volume for an assay: >200 mL E.coli culture volume or corresponding quantity. Protocol can be scaled up easily.
Protocol:	Purification of his-tagged protein in batch gravity flow or on FPLC columns
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 pellets corresponding to 200 mL bacterial culture on ice for 15 min. 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 10 mL Native Lysis Buffer supplemented with 1 mg/mL lysozyme, and pour it into a 50 mL conical centrifuge tube.
3. If the solution is very viscous, add 3 units Benzonase® per mL E.coli culture volume to the lysis buffer. Alternatively or additionally, sonicate the lysate to improve cell disruption.
4. Incubate on an end-over-end shaker at room temperature for 30 min, or at 4 °C for 1 h, depending on the temperature stability of the protein.
5. Centrifuge the lysate for 30 min at 10,000 x g and 2-8 °C. Carefully collect the supernatant without touching the pellet. 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 Agarose by inverting the bottle until the suspension is homogeneous. Transfer 1 mL of the 50 % suspension (corresponding to 500 µL bed volume) to a 15 mL conical centrifuge tube. Allow the resin to settle by gravity and remove the supernatant. Tip: Alternatively, resin equilibration can be performed directly in the disposable gravity flow column.
7. Add 2.5 mL Native Lysis Buffer and gently resuspend the slurry to equilibrate the resin. Allow the resin to settle by gravity and remove 2 mL supernatant.
8. Add 10 mL cleared lysate to the equilibrated HighSpec Ni-NTA Agarose resin and incubate at 4 °C for 1 h on an end-over-end shaker. Tip: Alternatively, batch binding can be performed directly in a gravity flow column with closed bottom and top outlets.
9. Transfer the binding suspension to a disposable gravity flow column with a capped bottom outlet. Use Lysis Buffer to rinse the centrifuge tube and remove resin adhered to the wall.
10. Remove the bottom cap of the column and collect the flow-through.
11. Wash the column with 5 mL Native Wash Buffer. Repeat the washing step at least 3 times.
12. Elute the His-tagged protein 5 times using 0.5 mL Native Elution Buffer. Collect each eluate in a separate tube and determine the protein concentration of each fraction. Optional: Incubate the resin for 15 min in Elution Buffer before collecting the eluate to increase protein yields.
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.

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

1. Thaw the E. coli cell pellet on ice.
2. Resuspend the cell pellet in 10 mL Denaturing Lysis Buffer. Optional: Benzonase® can be added to the lysate to reduce viscosity caused by nucleic acids (3 U/mL bacterial culture). Nucleic acids can also 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. Resuspend the HighSpec Ni-NTA Agarose by inverting the bottle until the suspension is homogeneous. Transfer 1 mL of the 50% suspension (corresponding to 0.5 mL bed volume)

Application Details

into a 15 mL conical centrifuge tube. Allow the resin to settle by gravity and remove the supernatant.

6. Add the cleared lysate to the resin and incubate the mixture for 1 h at room temperature on an end-over-end shaker. Tip: Alternatively, batch binding can be done directly in a gravity flow column with closed top and bottom outlet.
7. Transfer the binding suspension to a disposable gravity flow column with a capped bottom outlet. Use Lysis Buffer to rinse the centrifuge tube and remove resin adhered to the wall.
8. Remove the bottom cap of the column and collect the flow-through.
9. Wash the column with 5 mL Denaturing Wash Buffer. Repeat the washing step at least 3 times.
10. Elute the His-tagged protein 5 times using 0.5 mL Denaturing Elution Buffer. Collect each eluate in a separate tube and determine the protein concentration of each fraction. Tip: If the target protein is acid-labile, elution can be performed with 250-500 mM imidazole.
11. 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.
12. Optional: Perform Western Blot experiment using PentaHis Antibody.

Calculation of Results:	Analyze by SDS-PAGE, Bradford Assay or spectrophotometrically.
Assay Precision:	4.5 h
Restrictions:	For Research Use only

Handling

Format:	Liquid
Storage:	4 °C

Images

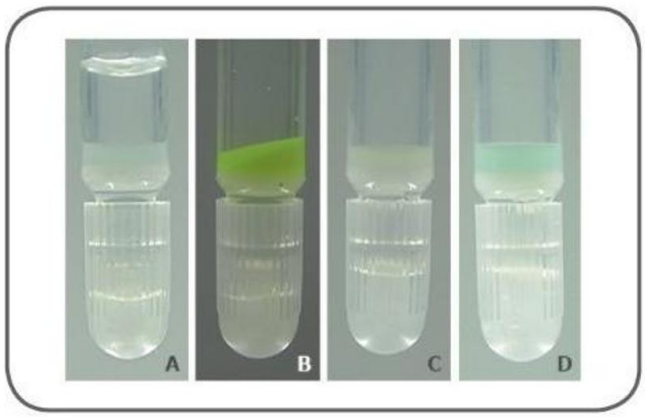

Image 1. Fig. 4: PureCube Ni-NTA Agarose is robust against oxidation and regenerable. PureCube Ni-NTA Agarose was exposed to 5mM DTT for 1 h (A). After demonstrating that it could still bind GFP (B), the resin was washed, stripped (C), and reloaded with Ni2+ (D) following standard Cube protocol (see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

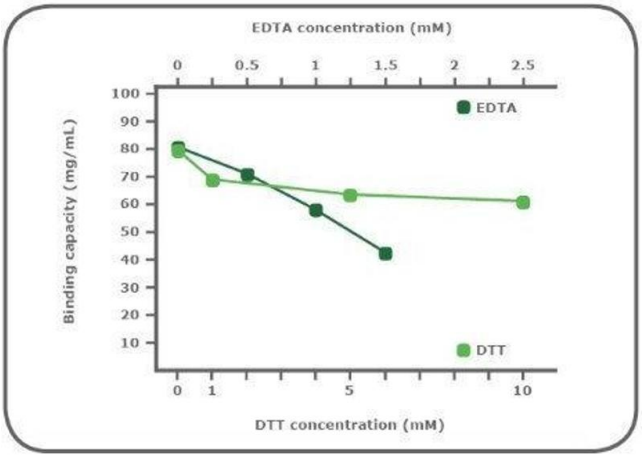

Image 2. Fig. 2: Over 20% more yield obtained with PureCube Ni-NTA Agarose. SDS-PAGE of GFP expressed in E. coli and purified in gravity columns with PureCube Ni-NTA Agarose and Ni-NTA resin from Competitor Q. 80 mg/mL protein yield was obtained with PureCube Ni-NTA Agarose (E1–E4, Cube) compared to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

SDS-PAGE

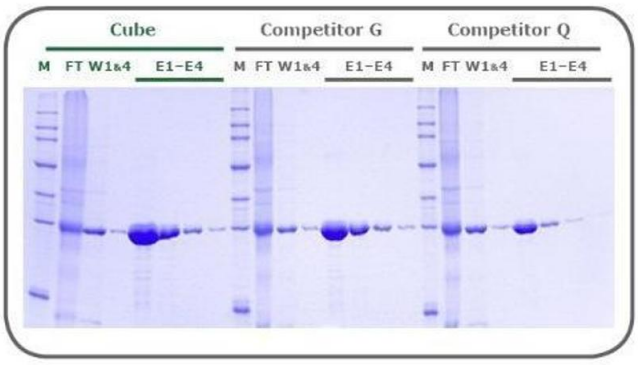

Image 3. Fig. 3: NTA is robust in the presence of reducing and chelating agents. GFP-His was purified on gravity columns containing PureCube Ni-NTA Agarose after exposing the resin for 1 h to 3 concentrations of DTT or EDTA. NTA exhibits a shallow decay rate in binding capacity.

Please check the product details page for more images. Overall 4 images are available for ABIN3199239.