Datasheet for ABIN4368209

INDIGO Ni-Agarose

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Overview

Quantity:	10 mL
Application:	Purification (Purif)

Product Details

Purpose:	Specific binding and purification of his-tagged proteins
Brand:	HighSpec
Sample Type:	Cell Culture Supernatant, Cell Lysate, Tissue Lysate
Specificity:	Affinity to His-tagged proteins
Characteristics:	• Novel His affinity ligand providing stability in 20 mM EDTA and 20 mM DTT • Binding capacity up to 80 mg/mL • Suitable for high flow rates in batch and FPLC • pH tolerant from pH 4-9. • Delivered as 20 mL of a 50 % suspension • Average agarose bead size: 100 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

Product Details

	• End-over-end shaker • SDS-PAGE buffers, reagents and equipment Optional: Western Blot reagents and equipment
Bead Ligand:	Ni-INDIGO
Bead Matrix:	Agarose beads
Bead Size:	100 µm

Application Details

Comment:	Sample Volume for an assay: >200 mL E.coli culture volume or corresponding quantity. Protocol can be scaled up easily.
Sample Volume:	200 mL
Assay Time:	4 - 5 h
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.

Application Details

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) 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.
Restrictions:	For Research Use only

Handling

Format:	Liquid
Storage:	4 °C

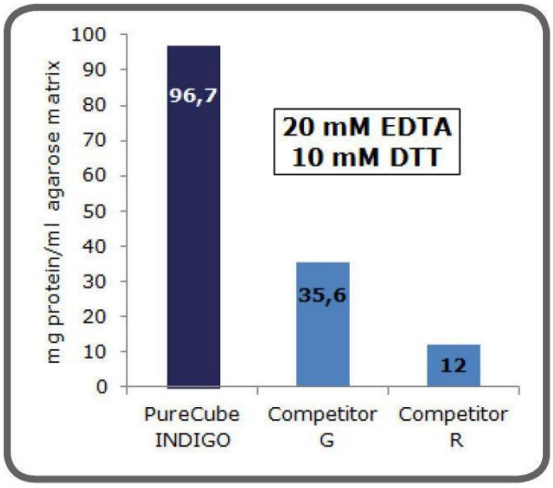


Image 1. Fig.2: PureCube 100 INDIGO Ni-Agarose outperforms competitor products. His-tagged GFP was purified on PureCube 100 INDIGO Ni-Agarose and two leading competitor matrices. Yields obtained with the INDIGO matrix were considerably higher at comparable purity. Buffer conditions: Sodium phosphate buffer pH 7.4, 10 mM DTT, 20 mM EDTA. Imidazole concentrations: Binding step: 10 mM, Wash: 20 mM, Elution: 250 mM. Fig.3: PureCube 100 INDIGO Ni-Agarose can be re-used multiple times without regeneration. GFP was spiked into E.coli lysates and purified in eight aliquots on the same 1 ml column filled with PureCube 100 INDIGO Ni-Agarose. Between each run, the column was briefly washed with loading buffer containing PBS and 10 mM imidazole. No decrease in performance was observed, even after eight consecutive runs. Left: Chromatogram; Right: SDS-PAGE. M: Marker, L: Lysate, S: Lysate spiked with GFP.

SDS-PAGE

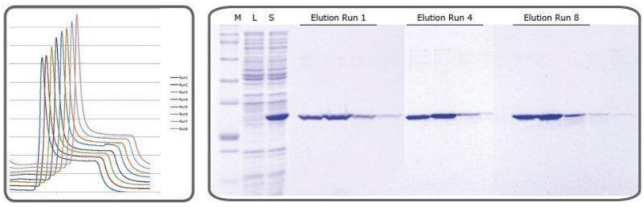
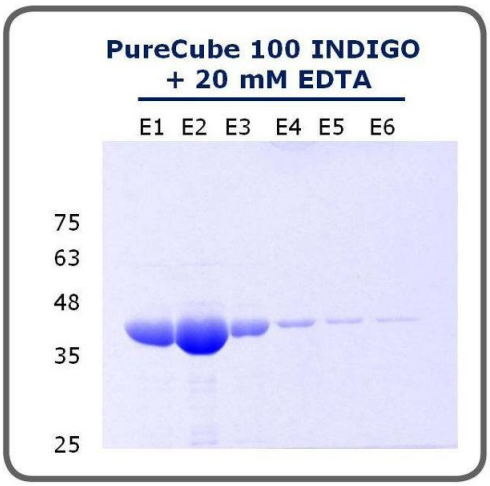


Image 2. Fig.3: PureCube 100 INDIGO Ni-Agarose can be re-used multiple times without regeneration. GFP was spiked into E.coli lysates and purified in eight aliquots on the same 1 ml column filled with PureCube 100 INDIGO Ni-Agarose. Between each run, the column was briefly washed with loading buffer containing PBS and 10 mM imidazole. No decrease in performance was observed, even after eight consecutive runs. Left: Chromatogram; Right: SDS-PAGE. M: Marker, L: Lysate, S: Lysate spiked with GFP.



SDS-PAGE

Image 3. Fig.1: PureCube 100 INDIGO Ni-Agarose is compatible with 20 mM EDTA and 20 mM DTT. SDS-PAGE of GFP expressed in E.coli and purified with PureCube 100 INDIGO Ni-Agarose in the presence of 20 mM EDTA and 20 mM DTT. High yield (>80 mg/ml) and purity were obtained.