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ab270529 His-Tag Protein Purification Column (Pre-packed, 5 x 5ml) - AminTrap

A product of Expedeon, an Abcam company

Applicable to Expedeon product codes TNN50005.

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www.abcam.com/ab270529

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This product is for research use only and is not intended for diagnostic use.

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1. Overview

His-Tag Protein Purification Column (Pre-packed, 5 x 5ml) - AminTrap (ab270529) is a simple and easy ready to use chromatography medium for purifying 6xHis-tagged proteins that have been expressed in series of expression hosts, such as *E. coli*., yeast, insect cells and mammalian cells.

The Ni-NTA resin contained in the column consists of 90 µm beads of highly cross-linked 6% agarose, to which Nitilotriacetic acid (NTA) has been coupled. The chelating group has then been charged with nickel ions (Ni^{2+}). This form is very stable octahedral structure of nickel ions in the center, which can protect the nickel ions from attack of the competitive small molecule. The structure of Ni-NTA is compatible with a certain concentration of reducing agents, denaturing agents, detergents and other additives.

His-Tag Protein Purification Column - AminTrap is one of a range of prepacked, ready-to-use columns for affinity chromatography. It is packed with Ni-NTA resin and available in 1 mL and 5 mL. The column has a standard interface and can be adapted to all kinds of chromatography system.

2. Materials Supplied and Storage

Store at +4°C immediately on receipt.

Item	Quantity	Storage temperature
His-Tag Protein Purification Column - AminTrap	Pre-packed, 5 x 5 mL	+4°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Lysis buffer (see Table 1 or 2 in Section 5)
- Wash buffer (see Table 1 or 2 in Section 5)
- Elution buffer (see Table 1 or 2 in Section 5)

4. Technical Considerations

4.1 Characteristics of the Ni-NTA Resin:

Column size	1.6 × 2.5 cm (5 mL)
Matrix	Highly cross-linked 4% agarose
Binding capacity	>40 mg 6xHis-tagged protein/mL medium
Particle size	45 – 165 µm
Maximum pressure	0.3 MPa, 3 bar
Storage Solution	1x PBS containing 20% ethanol

4.2 Chemical compatibilities of Ni-NTA Resin:

Reagent	Stability
Reductants	5 mM DTE 0.5-1.0 mM DTT 20 mM β-mercaptoethanol 5 mM TCEP 10 mM Reduced glutathione
Denaturants	8 M urea 6 M Guanidine -HCl
Detergents	2% Triton X-100 (non-ionic) 2% Tween 20 (non-ionic) 2% NP-40 (non-ionic) 2% cholate (anionic) 1% CHAPS (zwitterionic)
Other additives	500 mM imidazole 20% ethanol 50% glycerol 100 mM Na_2SO_4 1.5 M NaCl 1 mM EDTA 60 mM citrate
Buffers	50 mM sodium phosphate, pH 7.4 100 mM Tris-HCl, pH 7.4 100 mM Tris-acetate, pH7.4 100 mM HEPES, pH 7.4 100 mM MOPS, pH 7.4 100 mM sodium acetate, pH 7.4

5. Reagent Preparation

The basic principle of the following recommended buffer and other buffer is low concentration of imidazole in Lysis and Wash buffer and high in Elution buffer. Water and chemicals used for buffer preparation should be of high purity. It is recommended filtering the buffers by passing them through a 0.22 µm or 0.45 µm filter before use. His-Tag Protein Purification Pre-packed Column - AminTrap can be used for the his-tagged protein purification under native conditions and denaturing conditions, which need different buffer (see table 1 and table 2).

Table 1: Recommended buffer for native conditions.

Buffer	Volume	Ingredients
Lysis buffer	1 L	50 mM NaH_2PO_4 (7.80 g $NaH_2PO_4 \bullet 2H_2O$) 300 mM NaCl (17.54 g NaCl) 10 mM imidazole (0.68 g imidazole) Adjust the buffer pH to 8.0 with NaOH solution
Wash buffer	1 L	50 mM NaH_2PO_4 (7.80 g NaH2PO4•2H2O) 300 mM NaCl (17.54 g NaCl) 20 mM imidazole (1.36 g imidazole) Adjust the buffer pH to 8.0 with NaOH solution
Elution buffer	1 L	50 mM NaH_2PO_4 (7.80 g NaH2PO4•2H2O) 300 mM NaCl (17.54 g NaCl) 250 mM imidazole (17.0 g imidazole) Adjust the buffer pH to 8.0 with NaOH solution

Table 2: Recommended buffer for denaturing conditions.

Buffer	Volume	Ingredients
Lysis buffer	1 L	8 M Urea (480.50 g urea) 100 mM NaH_2PO_4 (15.60 g $NaH_2PO_4 \cdot 2H_2O$) 100 mM Tris•HCl (15.76 g Tris•HCl) Adjust the buffer pH to 8.0 with HCl solution
Wash buffer	1 L	8 M Urea (480.50 g urea) 100 mM NaH_2PO_4 (15.60 g $NaH_2PO_4 \cdot 2H_2O$) 100 mM Tris•HCl (15.76 g Tris•HCl) Adjust the buffer pH to 6.3 with HCl solution
Elution buffer	1 L	8 M Urea (480.50 g urea) 100 mM NaH_2PO_4 (15.60 g $NaH_2PO_4 \cdot 2H_2O$) 100 mM Tris•HCl (15.76 g Tris•HCl) Adjust the buffer pH to 4.5 with HCl solution

6. Sample Preparation

6.1 Recombinant native protein expressed in *E. coli* or yeast:

6.1.1 Single colonies were cultured in LB medium. According to the instruction, adding the inducers for a period of time.

6.1.2 Harvest cells from an appropriate volume of bacterial culture by centrifugation at 7,000 rpm for 10-15 minutes at +4°C. Discard supernatant and determine weight of pellet. Resuspend pellet in 1:10 ration (w/v) in lysis buffer and add lysozyme (0.2-0.4 mg/mL cell paste, if the host cell contains pLysS or pLysE, it can be without lysozyme) and PMSF (1 mM/mL cell paste).

6.1.3 If there is a high concentration of cell suspension, it is recommended to add 10 µg/mL RNase A and 5 µg/mL DNase I. Sonicate the cell suspension/lysate on ice.

6.1.4 Centrifuge the homogenized lysate at 10,000 rpm for 20 minutes at +4°C to clarify sample. Save supernatant.

6.2 Native protein expressed in yeast, insect or mammalian cells:

6.2.1 Harvest the cells from an appropriate volume of culture by centrifugation at 5,000 rpm for 10-15 minutes at +4°C. Save supernatant. If the supernatant does not contain EDTA, histidine and reductant, it can be purified directly, otherwise it will need dialysis to 1X PBS at +4°C.

6.2.2 For a large volume of supernatant, precipitation by adding ammonium sulfate and dialysis to1X PBS under +4°C is recommended.

6.3 Inclusion bodies from *E. coli*:

6.3.1 Harvest cells from an appropriate volume of bacterial culture by centrifugation at 7,000 rpm for 10-15 minutes at +4°C. Discard supernatant and determine weight of pellet.

6.3.2 Resuspend pellet at a 1:10 ratio (w/v) with Lysis Buffer. Sonicate the cell suspension/lysate on ice.

6.3.3 Centrifuge the homogenized sample at 10,000 rpm for 20 minutes at +4°C to pellet the inclusions.

6.3.4 Resuspend pellet at a 1:10 ratio (w/v) with denaturing Lysis Buffer (containing 8M urea). Sonicate, as needed, to re-dissolve the pellet.

6.3.5 Analyze the concentration of the target protein and continue with purification protocols under denaturing conditions.

7. Purification Procedure

7.1 Sample Purification:

7.1.1 Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the provided connector), or pump tubing, 'drop to drop' to avoid introducing air into the column. Remove the snap off end at the column outlet.

7.1.2 Wash the column with 10 column volumes of binding buffer at 5 mL/min for 5 mL column.

7.1.3 Apply the sample, using a syringe fitted to the connector or by pumping it onto the column.

7.1.4 Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent.

7.1.5 Elute with 5 column volumes of elution buffer. Other volumes may be required if the interaction is difficult to break.

7.2 Analysis:

7.2.1 Identify the fractions containing the His-tagged protein. Use UV absorbance, SDS-PAGE, or western blot.

7.3 Regeneration Procedure:

A column used to purify protein from cell extract usually contains some soluble substances and cell debris that are nonspecifically absorbed onto the matrix. Cleaning-in-Place eliminates material not removed by regeneration and prevents progressive buildup of contaminants. If the column is to be reused, these contaminants should be cleaned from the column, as they were not completely removed during the sample clarification steps.

<u>Remove the strong hydrophobic binding protein, lipoprotein and lipid:</u>

- Wash the column using 5-10 column volumes 30% isopropanol contacting for 15-20 minutes. Alternatively, use 2CV acidic or alkaline solution containing detergents, for example, 0.1 M acetic acid solution containing 0.1-0.5% non-ionic detergent, contacting for 1-2 hours.
- Finally wash the column with 10CV distilled water.

Remove the proteins bound via ionic interaction:

- Wash the column with 1.5M NaCl solution contacting for 10-15 minutes.
- Finally wash the column with 10 column volumes distilled water.

8. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)