PRODUCT INFORMATION

Ni-NTA sepharose Purification kit

For mammalian expression system

v. 230201

Catalog number	C07008-K01 / C07008-K02
Package	5 rxns / 10 rxns
Description	Affinity purified Ni-NTA is conjugated to NHS-sepharose. It is an efficient technique for isolating recombinant proteins or other proteins. The Ni-NTA sepharose is specifically designed for the purification of recombinant proteins fused to the 6 x histidine (6 x His) tag expressed in bacteria, insects, and mammalian cells. The sepharose is high affinity and selectivity for recombinant fusion proteins that are tagged with six tandem histidine residues. The Ni-NTA resin can be used to purify 6 x His tagged proteins under native and denaturing conditions. Proteins bound to the resin can be eluted with low pH buffer or competition with imidazole or histidine.

Component

Reagents & Materials	Quantity for 10 rxns (C07008-K02)	Quantity for 5 rxns (C07008-K01)	composition
Ni-NTA sepharose	2 mL X 1 vial	1 mL X 1 vial	50% slurry of Ni-NTA sepharose in 1X Phosphate Buffered Saline
Loading Buffer	50 mL X 1 vial	25 mL X 1 vial	20 mM sodium phosphate; 500 mM NaCl, pH 7.4
Wash Buffer	50 mL X 1 vial	25 mL X 1 vial	20 mM sodium phosphate; 500 mM NaCl; 20 mM imidazole, pH 7.4
Elution Buffer	10 mL X 1 vial	5 mL X 1 vial	20 mM sodium phosphate; 500 mM NaCl; 500 mM imidazole, pH 7.4
spin column	10 pcs	5 pcs	
collection tube	10 tubes	5 tubes	

Note: Sepharose is 1:1 suspension in Phosphate Buffered Saline, pH 7.4, containing 0.05% sodium azide as a preservative.

Product capacity	The binding and elution capacity of 1 mL settled Ni-NTA sepharose are commonly more than 5 mg of 6 x His fusion protein. Trying different wash buffers and elution buffers for optimal results is recommended.
Materials Required but Not Provided	• Micro-centrifuge capable of 15,000 x g • 1.5 mL Centrifuge tubes

	• End-over-end rotator • CoIP Lysis Buffer (mild reaction): 10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA; 0.5% NP-40 • RIPA (vigorous reaction): 100 mM Tris/Cl pH 7.5; 300 mM NaCl; 0.2% Sodium Deoxycholate (or 0.1% SDS); 2% NP-40
Storage	For sustainable use and long term storage, store at 2 °C to 8 °C. **DO NOT FREEZE.**
Precautions and Disclaimer	This product is for R&D use only, not for drug, household, or other uses.
Procedure	General notes The Ni-NTA sepharose is stored in Phosphate Buffered Saline containing 0.05% sodium azide. The PBS must be removed before use and the resin should be equilibrated with 1X Loading Buffer. The equilibration can be performed at room temperature or at 2-8 °C. In the case of bulk reaction. Users can make a pre-reaction through mixing sample and sepharose in 15 mL/ 50 mL tube, and then transfer the mixture into the column for binding. Suggestions on purification of 6 x His fusion protein 1. Cellular debris and particulate matter must be removed by centrifugation or filtration prior to purification on the column. 2. Highly viscous samples which may contain chromosomal DNA or RNA should be sonicated or treated with nuclease to decrease the viscosity. 3. Perform all steps on ice. A. Sample preparation (Lysis of Mammalian Cells) 1. Detach the cells from the culture dish and collect the cell suspension into the centrifuge tube. 2. Centrifuge the cell suspension at 400 x g for 5 minutes to pellet the cells. Carefully remove and discard the supernatant. 3. Wash cells by re-suspending the cell pellet in ice-cold PBS. 4. Centrifuge the cell suspension at 400 x g for 5 minutes to pellet the cells. Carefully remove and discard the supernatant. 5. Add 200 µL of Lysis Buffer to the cell pellet and vortex. 6. Incubate the sample for 15 minutes on ice.

Procedure

7. Remove cell debris by centrifugation at 15,000 x g for 5 minutes at 4°C.

B. Column preparation

1. Place an empty spin column on the collection tube.
2. Wash the column with 200 µL Loading Buffer.
3. Allow the buffer to drain from the column and leave residual Loading Buffer in the column to aid in packing the Ni-NTA sepharose, then discard the buffer in the collection tube.

C. Packing the column

1. Completely suspend the vial of Ni-NTA sepharose.
2. Transfer 200 µL volume to an empty centrifuge tube, and wash the sepharose with 1 mL Loading buffer.
3. Spin down the sepharose with 100 x g, 30 seconds' centrifugation and discard supernatant.
4. Immediately transfer the sepharose to the spin column. Allow the sepharose bed to settle. Please prevent the sepharose bed from getting dried.
   (Note: Make sure the column filter is fixed in the correct position before transferring the sepharose).

D. Binding 6 x His fusion protein to the column

1. Dilute the sample with Loading Buffer in 1:3 proportion.
2. Load the sample on the spin column and centrifuge the column at 100 x g for 30 seconds. Users can also perform this binding reaction in a new 1.5 mL centrifuge tube.
   (Note: Depending upon the 6 x His fusion protein and the flow rate, not all of the protein may bind. Repeat loading the sample to increase binding efficiency).
3. Collect the fractions using empty centrifuge tube.
4. Wash the spin column with 300 µL Wash Buffer more than 6 times.
   (Note: To eliminate the noisy band in sample, more washing step is recommended)

E. Elution of 6 x His fusion protein

1. Add 5 x 100 µL Elution Buffer to elute the bound 6 x His fusion protein from the spin column to the collection tube. This step can be supported by a

centrifugation at 100 x g for 30 seconds.

2. Assay sample concentration by measuring the absorbance at 280 nm and combine the fractions with highest absorbance.
   (Note: Measuring the absorbance after each Elution move can help collecting the sample more accurately).

F. Optional instead of elution step

Resuspended Ni-NTA sepharose in 100 µL 2 x SDS-Sample Buffer for 10 minutes at 95°C to dissociate immune-complexes from Ni-NTA sepharose. Ni-NTA sepharose can be collected by centrifugation at 2500 x g for 2 minutes at 4°C and SDS-PAGE is performed with the supernatant.

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