Ni-NTA Sepharose Purification Kit

Catalogue Number LDG0016RD

For Research Use Only. Not for use in diagnostic and therapeutic procedures.

RUO

Store at 2-8℃

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Leadgene® Ni-NTA Sepharose Purification Kit

1. Introduction

Affinity purified Ni-NTA is conjugated to NHS-sepharose. It is an efficient technique for isolating recombinant proteins or other proteins. The Ni-NTA resin can be used to purify 6 x His tagged proteins under native and denaturing conditions.

2. Test principle

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. Proteins bound to the resin can be eluted with low pH buffer or competition with imidazole or histidine.

3. Reagents provided and reconstitution

Reagents (Store at 2-8℃)	Quantity for 5 rxns (LMRD016005C040)	Quantity for 10 rxns (LMRD016010C040)	Composition	Reconstitution
Ni-NTA sepharose	1 vial (1 mL)	1 vial (2 mL)	50% slurry of Ni-NTA sepharose in 1X PBS	The PBS must be removed before use and the resin should be equilibrated with 1X Loading Buffer.
Loading Buffer (10X concentration)	1 vial (25 mL)	1 vial (50 mL)	20 mM sodium phosphate; 500 mM NaCl, pH 7.4	Dilute the loading buffer to 1X with distilled water.
Wash Buffer	1 vial (25 mL)	1 vial (50 mL)	20 mM sodium phosphate; 500 mM NaCl; 20 mM imidazole, pH 7.4	-
Elution Buffer	1 vial (5 mL)	1 vial (10 mL)	20 mM sodium phosphate; 500 mM NaCl; 500 mM imidazole, pH 7.4	Ready for use
spin column	5 pcs	10 pcs	-	-
collection tube	5 tubes	10 tubes	-	-

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4. Materials required but not provided

1. High quality distilled water
2. 5 µL to 1000 µL adjustable single-channel micropipettes with disposable tips
3. Micro-centrifuge capable of 15,000 x g
4. 1.5 mL microcentrifuge tubes
5. CoIP Lysis Buffer (mild reaction): 10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5mM EDTA; 0.5% NP-40
6. RIPA (vigorous reaction): 100 mM Tris/Cl pH 7.5; 300 mM NaCl; 0.2% Sodium Deoxycholate (or 0.1% SDS); 2% NP-40
7. Beakers, flasks, cylinders necessary for preparation of reagents
8. Timer
9. Clean paper towels
10. Disposable gloves
11. Discard container for bio-medical waste

5. Reagent preparation

- **Working wash buffer (1 X):** Dilute 1 volume of **10 X wash buffer** with 9 volumes of distilled water and homogenize by using micropipette.

6. Storage of reagents

- Before opened or reconstituted, all kit reagents should be kept properly at 2-8℃.
- For sustainable use and long term storage, store at 2 °C to 8 °C. **DO NOT FREEZE.**
- All working reagents should be prepared freshly and used on the same day.
- Alterations in physical appearance of kit components may indicate instability or deterioration.
- To minimize protein degradation, protease inhibitor cocktails are highly recommended.

7. Precautions & warnings

In order to obtain reproducible test results, the following rules should be strictly obeyed:

- All reagents and specimens should be considered as potentially hazardous. We therefore recommend that this product is handled by those persons who have been properly trained.

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- Wear suitable protective clothing and disposable gloves.
- Care should be taken to avoid reagents contacting with skin or eyes. If contacted, wash immediately and thoroughly with plenty of clean water.
- The assay should be performed as outlined in this manual, and in accordance with all instructions.
- Do not use expired or damaged products.
- This product is intended for *Research use only* and is not for use in diagnostic and therapeutic procedures.
- Do not mix or substitute reagents with those from different lots or other sources.
- Thoroughly and gently mix all the reagents and specimens prior to use.
- Use disposable graduated pipettes and tips to avoid cross-contamination of reagents or specimens which may invalidate the test.
- After use, all the reagents and specimens should be regarded as medical waste with risk of biological infection and properly disposed of in accordance with national regulations.

8. Procedure

- The Protein G 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 Wash 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.
- 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.
- Cellular debris and particulate matter must be removed by centrifugation or filtration prior to purification on the column.
- Highly viscous samples which may contain chromosomal DNA or RNA should be sonicated or treated with nuclease to decrease the viscosity.
- Perform all steps on ice.

<u>Purification of 6 x His fusion protein</u>

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

- **Column preparation**

(1) Place an empty spin column on the collection tube.
(2) Wash the column with 200 µL Loading Buffer.

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(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.

- **Packing the Column**

- (Note: Make sure the column filter is fixed in the correct position before transferring the sepharose).

(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 for 30 seconds 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.

- **Binding 6 x His fusion protein to the column**

(1) Dilute the sample with Wash Buffer in 1:3 proportion.

(2) Load the sample on the 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.

(1) (Note: Depending upon 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.

(2) (Note: To eliminate the noisy band in sample, more washing step is recommended).

- **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.

- (note: Depending on various characteristic of bound proteins, users can try altering the glycine Elution Buffer with guanidine-HCl Elusion Buffer).

(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).

- **Optional instead of elution step**

(1) Resuspended Ni-NTA sepharose in 100 µL 2 x SDS-Sample Buffer for 10 minutes at 95°C to dissociate the complexes from Ni-NTA sepharose.

(2) 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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