Ni-TED™

Protein Purification

Ni-TED Spin Columns, 25 reactions	Catalog # 97025
Ni-TED Spin Columns, 100 reactions	Catalog # 97100
Ni-TED Spin Columns, 25 reactions w/ solutions	Catalog # 98025
Ni-TED Spin Columns, 100 reactions w/ solutions	Catalog # 98100
Ni-TED Silica, 5 columns	Catalog # 97005
Ni-TED Silica, 10 columns	Catalog # 97020
Ni-TED Silica, 1 g	Catalog # 97001

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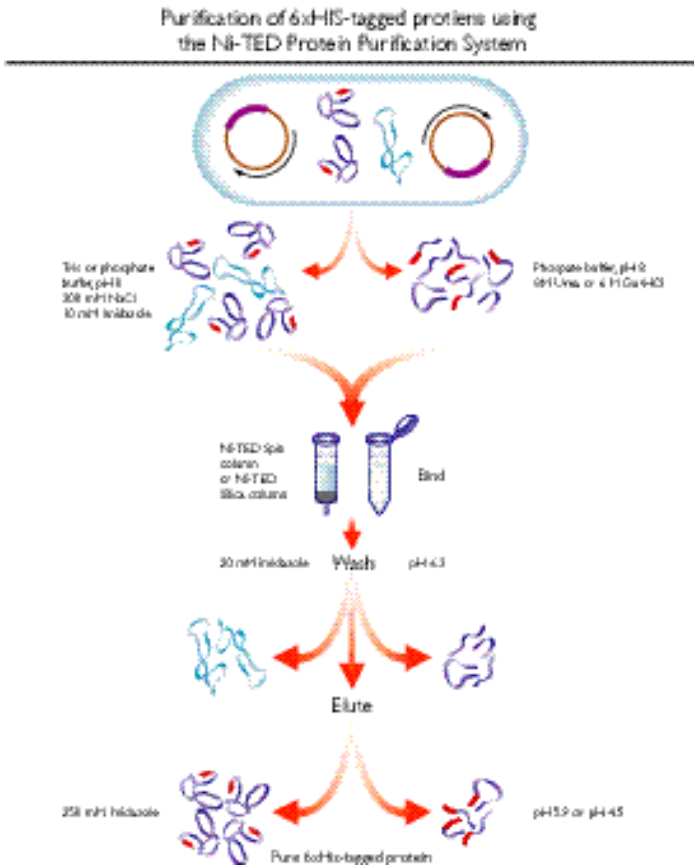
INTRODUCTION

The purification of 6xHis-tagged proteins consists of 4 steps: cell lysis, binding, washing, and elution (see flowchart). Purification of recombinant proteins using the Ni-TED system does not depend on the 3-dimensional structure of the protein or 6xHis tag. This allows one-step protein purification under either native or denaturing conditions, from dilute solutions and crude lysates. Strong denaturants and detergents can be used for efficient solubilization and purification of receptors, membrane proteins, and proteins that form inclusion bodies. Reagents that allow efficient removal of nonspecifically binding contaminants can be included in wash buffers. Purified proteins are eluted under mild conditions by adding 100–300 mM imidazole as a competitor or by a slight reduction in pH.

FLOWCHART



Ni-TED SPIN KITAND COLUMNS

The Ni-TED Protein Purification System is designed for one-step purification of 6xHis-tagged proteins by metal-chelate affinity chromatography. The Ni-TED system can be used to purify 6xHis-tagged proteins from any expression system including baculovirus, mammalian cells, yeast, and bacteria.

For fast, small-scale purification of 6xHis-tagged proteins

Binding capacity: 50 µg/spin column (2.5 nmol @ ~20 kDa)
Support: Macroporous TED-silica membrane
Culture Volume: 0.1-10 ml
Min. Elution Vol.: 50 µl

Features and benefits

- One-step purification from crude lysate to >95% pure protein
- High binding affinity and high capacity
- Choice of purification under native or denaturing conditions
- Precharged, ready-to-use matrices

KIT CONTENTS

Component	Amount / 25 reactions	Amount / 100 reactions	Composition	Storage
Resuspension Buffer	45 ml	175 ml	50 mM Tris, pH 8.0 300 mM NaCl 10 mM Imidazole	Room Temp.
Lysozyme, lyophilized	45 mg	2 x 45 mg		-20˚C
Buffer A	33 ml	125 ml	50 mM Tris, pH 8.0 300 mM NaCl 20 mM Imidazole	Room Temp.
Elution Buffer	11 ml	50 ml	50 mM Tris, pH 8.0 300 mM NaCl 250 mM Imidazole	Room Temp.
Spin-Columns	25	100		Room Temp.
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BEFORE STARTING

Resuspend each 45 mg aliquot of lysozyme in 450 µl of sterile water. The concentration of the lysozyme is now 100 mg/ml. Once resuspended, store the lysozyme at –20˚C.

NOTE: Save 30 µl of each fraction for SDS PAGE analysis.

Basic Protocol

1. Resuspend the cell pellet from 0.1-10 ml of culture in 0.8 ml of Resuspension Buffer. Add 8 µl of lysozyme.
2. Sonicate or homogenize cells by vortexing and spin for 10 minutes at 12,000 x *g*.
3. Equilibrate the Ni-TED spin-column with 600 µl of Resuspension Buffer (no lysozyme needed). Centrifuge for 2 minutes at 2,000 x *g*.
4. Load approximately 700 µl of cleared lysate onto the pre-equilibrated spin-column and centrifuge for 2 minutes at 2,000 x *g*. To add the remaining cleared lysate to the column, empty the collection tube and add the remaining 100 µl. Centrifuge for 2 minutes at 2,000 x *g*.
5. Wash the column twice with 600 µl of Buffer A. Centrifuge for 2 minutes at 2,000 x *g*.
6. To elute His-tagged protein, add 100 µl of Elution Buffer to the column and incubate the sample for 3 minutes at room temperature. Centrifuge for 2 minutes at 2,000 x *g*. Repeat twice more, each with 100 µl of Elution Buffer. Pool the eluted fractions, or alternatively analyze each fraction separately.

Analysis:

Save aliquots of the cleared lysate, the flow through, the washes and the elutions. These can be run out on SDS PAGE gels to determine the efficiency of the purification.

NOTE: A minimum elution volume of 50 µl may be used. This will yield a more concentrated purified protein.

NOTE: Triton X-100 may be added to the Resuspension Buffer to a final concentration of 0.2%. This may help resuspend the cell pellet, but will not affect the ability of the His-tagged protein binding to the spin column.

NI-TED SILICA PREPACKED COLUMNS

For fast, medium-scale purification of 6xHis-tagged proteins

Binding capacity: 900 µg/column (45 nmol @ ~20 kDa)
Support: Macroporous silica
Bead structure: Spherical, silica particles
Bead size: 35-70 µm
Form: Dry matrix packed in columns, precharged with Ni_2^+
Culture Volume: 20-100 ml
Min. Elution Vol.: 500 µl

Basic Protocol

The protocol for the Ni-TED Silica prepacked columns is very similar to the Ni-TED spin-columns, except that the solutions are allowed to drain through the column by gravity.

SOLUTIONS NEEDED:

1. **Resuspension Buffer** 50 mM Tris, pH 8.0, 300 mM NaCl, and 10 mM Imidazole, 0.2% Triton X-100
2. **Lysozyme** 100 mg/ml
3. **Wash Buffer** 50 mM Tris, pH 8.0, 300 mM NaCl, and 20 mM Imidazole
4. **Elution Buffer** 50 mM Tris, pH 8.0, 300 mM NaCl, and 250 mM Imidazole

NOTE: It is important to add Triton X-100 to a final concentration of 0.2% to the Resuspension Buffer. This will help the lysate to enter the silica column.

1. Resuspend the cell pellet from 20-100 ml of culture in 2-5 ml of Resuspension Buffer. Add lysozyme to a final concentration of 1 mg/ml.
2. Sonicate or homogenize cells by vortexing and spin for 10 minutes at 12,000 x *g*. Place the Ni-TED silica prepacked column in a 15 ml centrifuge tube.
3. Equilibrate the Ni-TED silica prepacked column with 2 ml of Resuspension Buffer (no lysozyme needed). Allow the column to drain by gravity.
4. Load up the cleared lysate onto the pre-equilibrated column and allow the column to drain by gravity.
5. Wash the column twice with 3 ml of Buffer A. Allow the column to drain by gravity.
6. To elute His-tagged protein, add 1 ml of Elution Buffer to the column. Allow the column to drain by gravity. The Ni-TED silica prepacked column fits well into a 1.5 or 2 ml microcentrifuge tube.

7. Repeat elution twice more with 1 ml of Elution Buffer. Pool the eluted fractions, or alternatively analyze each fraction separately.

Analysis:

Save aliquots of the cleared lysate, the flow through, the washes and the elutions. These can be run out on SDS PAGE gels to determine the efficiency of the purification.

Ni-TED SILICA

BATCH PROTOCOL

Using Ni-TED silica it is possible to purifying His-tagged fusion proteins using a batch-type purification procedure. The batch procedure entails binding the protein to the Ni-TED silica in solution and then packing the protein-resin complex into a column for the washing and eluting steps. This strategy promotes efficient binding of the His-tagged protein especially when the His-tag is not fully accessible or when the protein in the lysate is present at very low concentrations.

Washing and elution steps are identical for the batch and column procedure.

Use the following table to determine the amount of silica resin needed for a given amount of purified protein.

Amount of Ni-TED Silica	Approx. amount of protein*
0.3 g	1 mg
1 g	3 mg
1.5 g	4.5 mg
2 g	6 mg

* 1 g of Ni-TED silica will purify at least 3 mg of recombinant protein. Amount can vary depending on the protein.

The following procedure allows you to batch bind a 5 ml aliquot of lysate containing your His-tagged protein to the Ni-TED silica.

SOLUTIONS NEEDED:

1. **Resuspension Buffer** 50 mM Tris, pH 8.0, 300 mM NaCl, and 10 mM Imidazole, 0.2% Triton X-100
2. **Lysozyme** 100 mg/ml
3. **Wash Buffer** 50 mM Tris, pH 8.0, 300 mM NaCl, and 20 mM Imidazole
4. **Elution Buffer** 50 mM Tris, pH 8.0, 300 mM NaCl, and 250 mM Imidazole

Basic Protocol

1. Spin down the cells containing the recombinant protein and resuspend the cell pellet in 5 ml of Resuspension Buffer. Add lysozyme to a final concentration of 1 mg/ml.
2. Sonicate or homogenize cells by vortexing and spin for 10 minutes at 12,000 x *g*. Collect the cleared lysate.
3. Weigh out 0.3 g of Ni-TED silica and resuspend in 2 ml of Resuspension Buffer.
4. Add this resuspended resin to the 5 ml of cleared lysate.
5. Transfer the resin/lysate slurry to an empty chromatography column.
6. Gently rock the column for 10 minutes to 1 hour* to allow the recombinant protein to fully bind.
7. Let the resin settle by gravity flow and collect the flow through, (unbound protein).
8. Wash the column twice with 4 ml of Wash Buffer. Collect the washes separately, for gel analysis.
9. Elute the protein by applying 4 ml of Elution Buffer.
10. Collect 1 ml fractions.
11. Monitor the elutions by A_{280} readings of the fractions.
12. Pool the fractions that contain the peak absorbance and concentrate them by standard methods. Alternatively, you may decide to assay the fractions by functional assay or by SDS-PAGE gels.

* The length of time for the optimum His-tagged protein binding to the Ni-TED silica in batch mode will vary from protein to protein.

Analysis:

Save aliquots of the cleared lysate, the flow through, the washes and the elutions. These can be run out on SDS PAGE gels to determine the efficiency of the purification.

BUFFERS FOR PURIFICATION UNDER DENATURING CONDITIONS

NOTE: We have found that when using Ni-TED protein purification products that we in general get better results (a higher yield of purified protein) when His-tagged proteins are purified under native conditions than under denaturing conditions.

Lysis Buffer

Buffer A: 6 M Guanidine-HCl, 0.1 M NaH_2PO_4, 0.01 M Tris-Cl pH 8.0.

Buffer B: 8 M Urea, 0.1 M NaH_2PO_4, 0.01 M Tris-Cl pH 8.0.

Wash Buffer

Buffer C: 8 M Urea, 0.1 M NaH2PO4, 0.01 M Tris-Cl pH 6.3.

Elution Buffers

Buffer D: 8 M Urea, 0.1 M NaH_2PO_4, 0.01 M Tris-Cl pH 5.9.

Buffer E: 8 M Urea, 0.1 M NaH_2PO_4, 0.01 M Tris-Cl pH 4.5.

NOTE: Due to the dissociation of urea, the pH of Buffers B, C, D and E should be adjusted immediately prior to use. Do not autoclave.

Optimal purification parameters will vary for each fusion protein, and some experimentation may be necessary to obtain the level of purification desired. The procedures outlined in this manual are designed to provide initial parameters for purification. Further steps or development may be needed to obtain purified protein.

RELATED PRODUCTS

Media

LB Broth	Catalog #:	80020
LB Agar	Catalog #:	80040
Terrific Broth	Catalog #:	80060
SOB	Catalog #:	80080
SOC	Catalog #:	80100
Agar	Catalog #:	80120

Additives

Ampicillin	Catalog #:	80140
Kanamycin	Catalog #:	80160
Tetracycline	Catalog #:	80180
Tryptone	Catalog #:	80200
Yeast Extract	Catalog #:	80220
Casamino Acids	Catalog #:	80240
Glucose	Catalog #:	80260

RapidTrans (chemically competent *E. coli* cells)

- Consistent high quality cells available anytime
- >3 x 10^8 cfu/µg
- Economical pricing
- Adaptable to high throughput use
- Freedom to use anywhere from 1-96 reactions

TAM 1	Catalog #:	11096
TAM 1-F'	Catalog #:	10096

Active Motif guarantees the performance of this product when used as described in this Instruction Manual. The researcher must determine the suitability of this product for the intended use. If this product should fail to perform to your satisfaction for any reason other than misuse, please contact us.

Active Motif reserves the right to change, enhance, or upgrade the design, performance, or configuration of any product at any time. If any of our products should not meet with your expectations, please contact us.

We value and encourage the input of our customers. If you should have a comment or suggestion about any of our products or a new application idea or technique, please contact us.

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