His-Tagged Protein Purification Kit

Catalog Number IP050

For the purification of recombinant proteins containing a His-tag.

This package insert must be read in its entirety before using this product.

TABLE OF CONTENTS

Contents	Page
INTRODUCTION	2
PRINCIPLE OF THE ASSAY	2
LIMITATIONS OF THE PROCEDURE	3
REAGENTS	3
STORAGE	3
OTHER SUPPLIES REQUIRED	3
PRECAUTIONS	3
SAMPLE COLLECTION	4
REAGENT PREPARATION	4
TECHNICAL HINTS	4
PURIFICATION PROCEDURE	5
PURIFICATION PROCEDURE SUMMARY	6
ADDITIONAL ANALYSIS AND TYPICAL DATA	7
RECOVERY	8
CAPACITY	8
SPECIFICITY	8

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INTRODUCTION

With as many genes that are being discovered, especially by rapid genome sequencing, the pace of studying their protein products has to be increased dramatically in order to understand their biological functions. Obtaining pure proteins is a critical step in characterizing them biochemically. Purifying proteins is often challenging because of various modifications that can occur during post-transcriptional and post-translational processing.

To simplify and streamline the purification process, a successful approach has been to add "tags" to recombinant proteins during cloning and expression. One of the commonly used tags consists of six to ten consecutive histidine residues added to either the N- or C-terminus of the protein of interest. The His-tag allows the recombinant protein to be efficiently purified by immobilized metal affinity chromatography (IMAC). The short His-tag does not need to be removed from the recombinant protein because it usually has negligible effect on protein structure and function. This is in sharp contrast to those tags that consist of much larger proteins or protein fragments.

Even with a His-tag, purifying diverse proteins by IMAC still requires substantial effort. For example, there are often contaminants that have high affinity for immobilized metal ions, thus hindering purification. In addition, each protein behaves differently on such columns, creating difficulties in streamlining the purification processes of diverse proteins. To circumvent these problems, the His-tagged Protein Purification Kit provides a complete system designed to allow efficient and rapid purification of diverse proteins with a His-tag in a single step using a standardized protocol. The kit can be used to purify up to 20 different proteins at microgram quantities, amounts sufficient for further analysis such as N-terminal sequencing and mass spectroscopy.

PRINCIPLE OF THE ASSAY

This kit employs an immunopurification technique. The antibody beads are agarose matrices coupled with a mouse monoclonal antibody specific for a polyhistidine peptide epitope, thus non-specific interactions are greatly reduced. Samples containing the His-tag protein of interest are incubated with the antibody beads and then transferred to microcentrifuge filter units. After centrifugation, the antibody beads with bound molecules are retained in the filter units while non-bound materials are collected in microcentrifuge tubes. Molecules weakly bound to the beads are removed with Wash Buffer. The proteins of interest bound to the beads are recovered with Elution Buffer, and the pHs of the resulting solutions are adjusted with Neutralization Buffer.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.

REAGENTS

Antibody Beads (Part 840339) - 400 µL of 50% suspension of agarose beads containing a mouse monoclonal antibody specific for a polyhistidine peptide epitope, in 20% ethanol.

Positive Control (Part 840340) - 1 vial of conditioned media from NS0 mouse myeloma cells expressing a recombinant His-tagged protein, lyophilized.

2X PBS (Part 895532) - 2 vials (1.5 mL/vial) of concentrated solution with preservative.

Elution Buffer 1 (Part 845239) - 1.5 mL of 0.25 M acetic acid.

5X Wash Buffer (Part 895535) - 15 mL of concentrated solution with preservative.

Neutralization Buffer 1 (Part 845241) - 0.7 mL of 2 M Tris-HCl with preservative.

6X Sample Buffer (Part 845242) - 1 mL of concentrated non-reducing SDS-PAGE sample buffer.

Microcentrifuge Filters (Part 895538) - 1 bag containing 20 filter units with microcentrifuge tubes.

STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8° C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="8">Opened/ Reconstituted Reagents</td><td>Antibody Beads</td><td rowspan="4">May be stored for up to 1 year at 2 - 8° C.*</td></tr>
<tr><td>Elution Buffer 1</td></tr>
<tr><td>5X Wash Buffer</td></tr>
<tr><td>Neutralization Buffer 1</td></tr>
<tr><td>Positive Control</td><td>Store 0.5 mL aliquots for up to 1 year at -20° C.*</td></tr>
<tr><td>2X PBS</td><td rowspan="3">May be stored for up to 1 year at room temperature.*</td></tr>
<tr><td>6X Sample Buffer</td></tr>
<tr><td>Microcentrifuge Filters</td></tr>
</table>

*Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microcentrifuge and tubes.
- Pipettes and tips, including large orifice tips for pipetting Antibody Beads.
- Deionized or distilled water.
- Rocker or shaker.
- Vortex mixer.
- 100 mL graduated cylinder and container.

PRECAUTIONS

The Elution Buffer provided with this kit is a weak acid solution. Take necessary precautions.

SAMPLE COLLECTION

Samples should be at neutral pH (pH 6 - 8) and physiological salt concentration (0.1 - 0.25 M NaCl). If desired, samples can be concentrated prior to use by ultrafiltration or other means. Particulate matter should be removed from samples by centrifugation or filtration prior to performing the purification procedure.

REAGENT PREPARATION

Antibody Beads - Prior to use, invert the vial containing the antibody beads several times until the suspension is a homogeneous mix. Immediately pipet 20 µL of the 50% suspension of antibody beads, using a large orifice pipet tip, into 0.2 mL of 1X PBS in a microcentrifuge tube. Large orifice tips can be made by cutting the small end off regular pipet tips. Centrifuge for 1 minute at 4000 x g and remove the excess buffer without disturbing the beads. The volume of the washed beads is 10 µL.

1X PBS - Dilute 3 mL of 2X PBS with 3 mL of deionized or distilled water to make 6 mL of 1X PBS.

Positive Control - Reconstitute Positive Control in 1 mL of 1X PBS. If not used immediately, store 0.5 mL aliquots at -20° C.

Wash Buffer - Dilute 15 mL of 5X Wash Buffer with 60 mL of deionized or distilled water to make 75 mL of Wash Buffer.

SDS-PAGE Sample Buffer - For non-reducing SDS-PAGE, dilute 6X Sample Buffer 6-fold into the purified His-tagged protein preparations prior to electrophoresis. When electrophoresis under reducing conditions is desired, add a reducing agent such as β-mercaptoethanol. For use as an alternative elution solution, dilute the 6X Sample Buffer 6-fold in deionized or distilled water.

TECHNICAL HINTS

- A 1 hour incubation at room temperature is convenient for samples containing high levels of His-tagged proteins (greater than 100 ng/mL). For samples containing His-tagged proteins at low concentrations (10 - 100 ng/mL), incubation overnight at 2 - 8° C is recommended. For samples containing His-tagged proteins at extremely low concentrations (less than 10 ng/mL), concentrating the samples is recommended prior to the incubation.
- Optimal binding between the His-tagged proteins and antibody beads is dependent upon many factors such as pH (pH 6 - 8), salt content (0.1 - 0.25 M NaCl), and concentration of His-tagged proteins in the samples to be analyzed. Prior to use of this kit, it would be helpful to determine these factors. For example, Western blot or ELISA analysis can be used to estimate the concentration of His-tagged protein.

PURIFICATION PROCEDURE

1. Prepare Antibody Beads, Positive Control, 1X PBS, Wash Buffer and SDS-PAGE Sample Buffer as described in the Reagent Preparation section. Once the reagents are prepared, all further steps can be carried out at room temperature, unless indicated otherwise.

2. Add desired volume of sample to a tube containing 10 μL of washed Antibody Beads. The sample size will determine the size of tube to be used (microcentrifuge, 15 mL, 50 mL, or larger conical tubes). As a control, mix 0.5 mL of Positive Control with 10 μL of washed Antibody Beads in a microcentrifuge tube.

3. Mix the sample and beads by inverting the tube several times. Incubate for 1 hour at room temperature with gentle rocking or shaking.

4. Transfer the suspension to a Microcentrifuge Filter using a large orifice pipette tip.

5. Centrifuge for 30 seconds at 4000 x g. Discard the flow-through fraction or save for later analysis.

6. Add 600 μL of Wash Buffer to the filter. Resuspend the beads by vortexing briefly. Centrifuge for 30 seconds at 4000 x g. Discard the flow-through fraction. Repeat the wash step two more times.

7. Transfer the filter to a new tube containing 15 μL of Neutralization Buffer 1.

8. Add 35 μL of Elution Buffer 1 to the filter. Resuspend the beads by tapping the tube for 1 minute. Centrifuge for 30 seconds at 4000 x g. To ensure full recovery of the target protein from the beads, add an additional 10 μL of Elution Buffer 1 to the filter and repeat the elution process. Analyze immediately or store sample at -20° C or -80° C.

PURIFICATION PROCEDURE SUMMARY

1. Prepare reagents as instructed.

2. Add sample to a tube containing 10 µL of Antibody Beads and mix. Incubate 1 hour at RT on a rocker or shaker.

3. Transfer the suspension to a Microcentrifuge Filter. Centrifuge for 30 seconds at 4000 x g. Remove flow-through fraction.

4. Wash the beads 3 times with 600 µL of Wash Buffer. Centrifuge for 30 seconds at 4000 x g.

5. Transfer the filter to a new tube containing 15 µL of Neutralization Buffer 1.

6. Add 35 µL of Elution Buffer 1. Tap tube for 1 minute. Centrifuge for 30 seconds at 4000 x g.

7. Add additional 10 µL Elution Buffer 1 and repeat the elution process.

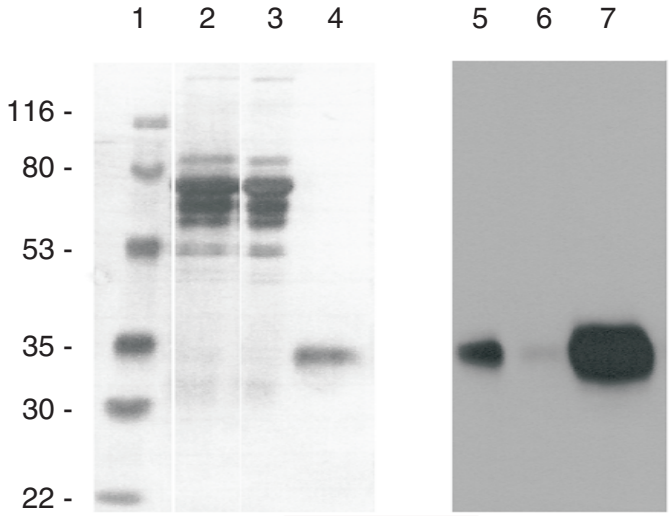
ADDITIONAL ANALYSIS AND TYPICAL DATA

The purified His-tagged proteins can be analyzed by western blot, silver staining of SDS-PAGE, N-terminal sequencing and mass spectroscopy. Amounts used in these analyses depend upon the concentrations of recovered material and sensitivities of techniques used. Typical data are shown below with the positive control included in the kit.

Performance Analysis of the kit with Silver Staining of SDS-PAGE and Western Blot
The Positive Control was prepared and processed using the recommended procedure as described for the kit. Aliquots of the Positive Control, saved at different steps of the procedure, were analyzed by SDS-PAGE. The proteins were detected by silver staining of SDS-PAGE gels. The His-tagged proteins were detected by western blot using HRP-labeled mouse anti-polyhistidine antibody (R&D Systems' Catalog # MAB050H).

Silver Staining of SDS-PAGE
Lane 1: Molecular weight markers (kDa) as indicated on the left of the figure.
Lane 2: Positive Control before purification.
Lane 3: Unbound material in the flow through fraction.
Lane 4: Purified Positive Control.

Western Blot
Lane 5: Positive Control before purification.
Lane 6: Unbound material in the flow through fraction.
Lane 7: Purified Positive Control.



RECOVERY

The recovery of His-tagged protiens depends on many factors, such as sample concentration and incubation time. By following the guidelines described in the previous sections, the recovery is greater than 80%.

CAPACITY

The antibody beads (20 μL of 50% suspension) can bind more than 4 μg of His-tagged protein.

SPECIFICITY

Following the standard protocol presented here, the antibody beads have been shown to recover only His-tagged proteins from crude mixtures.