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Dynabeads® His-Tag Isolation & Pulldown

Catalog nos. 10103D, 10104D, 10105D **Store at 2 to 8°C**

Rev. Date: October 2011 (Rev. 001)

Kit Contents

Product no.	Volume	Capacity
10103D	2 mL	40 tests
10104D	10 mL	200 tests
10105D	10 × 10 mL	2000 tests

Dynabeads® His-Tag Isolation & Pulldown contains 40 mg beads/mL in 20% ethanol and has a capacity of isolating 40 µg of a 28 kDa histidine-tagged protein/mg (25 µL) beads.

Product Description

Dynabeads® His-tag Isolation & Pulldown were developed for the isolation of histidine-tagged proteins. These Dynabeads® are coated in a cobalt-based Immobilized Metal Affinity Chromatography (IMAC) chemistry. The optimized chemistry on these Dynabeads® binds histidine-tagged proteins with higher selectivity compared to Agarose- and Sepharose-based bead systems. Dynabeads® magnetic bead-based technology makes the purification quick and easy:

Add Dynabeads® to a sample containing histidine-tagged proteins and allow the proteins to bind to the Dynabeads®. Isolated proteins can be left on the Dynabeads® and used directly in downstream applications. Alternatively, the isolated histidine-tagged proteins can be eluted from the beads.

Elution conditions are less stringent than other technologies thus yielding more functional isolated proteins. These characteristics make Dynabeads® the ideal product for purifying histidine-tagged proteins expressed in *E. coli*.

Required Materials

- Magnet (DynaMag™) for manual or automated protocols. See www.lifetechnologies.com/magnets for recommendations.
- Mixing device with tilting and rotation (e.g. HulaMixer® Sample Mixer).
- Test tubes and pipettes.
- Buffers (see Table 1).

General Guidelines

- There are many different ways of preparing a cell lysate containing expressed histidine-tagged proteins. It is important that the lysate does not contain EDTA (or other chelators), ionic detergents, DTT or DTE. A pH between 7 and 8 should be used. Alternative lysis strategies for *E. coli* can be used, such as Commercially available ready-made lysis buffers.
  - 1X Binding/Wash Buffer (see "Protocol") with1% Triton® X-100 (for mammalian and insect cells only).
  - French press
  - Sonication
    Efficiency of lysis can be increased by the addition of lysozyme.
- To avoid a sticky pellet, add DNase I.
- We generally recommend applying the tube to the magnet for 2 min, but the sample can be handled when the beads are visually collected at the tube wall and the liquid is clear.
- Other cell types than *E. Coli* (e.g. yeast or mammalian) can also be used for histidine-tagged protein expression, with some optimization of the purification protocol.
- Protocols for the purification of histidine-tagged proteins using other metal based IMAC technologies can easily be adapted for cobalt-based IMAC, with some optimization.

Table 1: Required buffers

2X Binding/Wash Buffer*	His Elution Buffer	2X Pull-down Buffer*	Buffer modifiers
• 100 mM Sodium-Phosphate, pH 8.0 • 600 mM NaCl • 0.02% Tween®-20	• 300 mM Imidazole • 50 mM Sodium-phosphate pH 8,0 • 300 mM NaCl • 0.01% Tween®-20	• 6.5 mM Sodium-phosphate, pH 7.4 • 140 mM NaCl • 0.02% Tween®-20	• 1 M NaCl • 0.1 M Imidazole

* Note that the 2X Binding/Wash Buffer and the 2X Pull-down Buffer need to be diluted to 1X concentration prior to use.

Alternative binding and/or washing buffers may also be used for isolation of your specific recombinant protein.

Protocol

Prepare your sample containing the histidine-tagged protein. Preferentially, you may prepare your sample in a total volume of 700 µL 1X Binding/Wash Buffer.

1. Thoroughly resuspend the Dynabeads® in the vial (vortex >30 sec or tilt and rotate 5 min).
2. Transfer 50 µL (2 mg) Dynabeads® to a microcentrifuge tube. Place the tube on a magnet for 2 min. Aspirate and discard the supernatant. Add your sample (prepared in Binding/Wash Buffer) to beads. Mix well.
3. Incubate on a roller for 5 min at room temperature (or colder if the protein is unstable at room temperature). The incubation time may be increased up to 10 min.
4. Place the tube on the magnet for 2 min, then discard the supernatant.
5. Wash the beads 4 times with 300 µL Binding/Wash Buffer by placing the tube on a magnet for 2 min and discard the supernatant. Resuspend the beads thoroughly between each washing step.
6. If the protein is to be eluted, proceed to step 7.
   - To use bead/protein complexes in another application, resuspend the bead/protein complex in a suitable volume of 1X Pull-down Buffer (or another buffer compatible with your downstream application).
   - If you wish to continue with a Pull-down, then continue to step 1 in "Protein Pull-down".
7. Add 100 µL His-Elution Buffer. Incubate the suspension on a roller for 5 min at room temperature (or colder if the protein is unstable at room temperature).
8. Apply on the magnet for 2 min and transfer the supernatant containing the eluted histidine-tagged protein to a clean tube.

Protein Pull-down

1. Prepare your sample in Pull-down Buffer in a total volume of up to 700 µL.
2. Add your sample (prepared in Pull-down Buffer) to the bead/protein complex from step 5 in "Protocol" on page 2.
3. Incubate on a roller for 10 min at room temperature (or cold if the protein is unstable at room temperature). The incubation time may be increased up to 30 min.
4. Place the tube on a magnet for 2 min, then discard the supernatant.
5. Wash the beads 4 times with 300 µL Binding/Wash Buffer by placing the tube on a magnet for 2 min and discard the supernatant. Resuspend the beads thoroughly between each washing step.
6. Add 100 µL His-Elution Buffer. Incubate the suspension on a roller for 5 min at room temperature (or cold if protein is unstable at room temperature). Collect the beads at the tube wall using a magnet and transfer the supernatant containing the eluted histidine-tagged protein and its interacting protein to a clean tube. The elution volume may be decreased to 50 µL.

Automated Purification Protocols

Protein purification using Dynabeads® His-tag Isolation and Pulldown can easily be automated on a wide variety of platforms. Automation protocols are available at: www.lifetechnologies.com.

Description of Materials

Dynabeads® His-tag Isolation and Pulldown are uniform, superparamagnetic beads, 1 µm in diameter, coupled with highly specific IMAC chemistry. The technology is comprised of a tetradentate metal chelator in which four of cobalt's six coordination sites are occupied. The imidazole rings of histidine residues present in a polyhistidine peptide chain are able to occupy the two remaining coordination sites, resulting in protein binding.

Related Products

Product	Cat. no.
DynaMag™-2	12321D
HulaMixer® Sample Mixer	15920D

REF on labels is the symbol for catalog number.

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