Cambio

Purification of His-tagged Proteins Under Native Conditions Using Cambio His Affinity MagBeads

Overview
This protocol describes the generation of a cleared lysate from an *E. coli* cell pellet and the subsequent purification of His-tagged proteins under native conditions using Cambio His Affinity MagBeads. Cambio offers a range of His Affinity MagBeads, including NTA and IDA materials loaded with nickel, cobalt, or other transition metals. Reagent amounts given apply to 10 mL IPTG-induced bacterial culture of a well-expressed protein (approximately 10–50 mg/L). Magnetic bead purification is easily scalable. To minimize unspecific binding and reduce cost, the volume magnetic bead suspension used should be adjusted to the expression level of interest. See Table 1 for more details.

In this protocol, cell lysis is done using lysozyme because it is an inexpensive and efficient method for cells that have been frozen. However, lysis methods using detergents (e.g., CHAPS) can also be used. The His-tagged target protein is purified from cleared lysate under native conditions in a bind-wash-elute procedure.
Magnetic beads are well-suited to purify proteins from dilute solutions, such as cell culture or medium supernatants. Please contact us if you have questions or need assistance optimizing a protocol for your application (support@cambio.co.uk).
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Please note that the concentration of our MagBeads has increased from 5% to 25%. Volumes given in this protocol reflect this change. Please discard previous versions of this protocol.

Equipment needed:

Ice bath
Refrigerated microcentrifuge (min 10,000 x g)
Micropipettor
Micropipetting tips
1.5 mL conical microcentrifuge tubes
Magnetic holder for microcentrifuge tubes (for separation of magnetic beads)
pH meter
End-over-end shaker
SDS-PAGE equipment
Optional: Western Blot equipment

Materials needed:

Cell pellet from expression screen (e.g., from 10 mL culture)
Cambio His Affinity MagBeads, e.g.
Cambio Ni-NTA MagBeads
Cambio Ni-IDA MagBeads
Cambio Co-NTA MagBeads

Reagents needed:

Sodium phosphate monobasic (NaH2PO4)
Sodium chloride (NaCl)
Imidazole
Sodium hydroxide (NaOH)
Lysozyme
Benzonase® nuclease (e.g. Merck Milipore, #707464)
Dithiothreitol (DTT)
Glycerol
Sodium dodecyl sulfate (SDS)
Bromophenol blue
Tris base
Hydrochloric acid (HCl)
Optional: Protease inhibitor cocktail (e.g. Roche cOmplete, #04693116001)
Optional: anti-His Antibody

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
NaH_2PO_4	50 mM	119.98	0.5 M	29.99 g/ 500 mL	5 mL
NaCl	300 mM	58.44	5 M	146.1 g/ 500 mL	3 mL
Imidazole	10 mM	68.08	1 M	6.8 g/ 100 mL	0.5 mL
Instructions: Mix in 40 mL water. Adjust the pH to 8.0 using NaOH and then add water to a total volume of 50 mL. Always prepare fresh.					

Wash Buffer, 50 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
NaH_2PO_4	50 mM	119.98	0.5 M	29.99 g/ 500 mL	5 mL
NaCl	300 mM	58.44	5 M	146.1 g/ 500 mL	3 mL
Imidazole	20 mM	68.08	1 M	6.8 g/ 100 mL	1 mL
Instructions: Mix in 40 mL water. Adjust the pH to 8.0 using NaOH and then add water to a total volume of 50 mL. Always prepare fresh.					

Elution Buffer, 50 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
NaH_2PO_4	50 mM	119.98	0.5 M	29.99 g/ 500 mL	5 mL
NaCl	300 mM	58.44	5 M	146.1 g/ 500 mL	3 mL
Imidazole*	500 mM	68.08	1 M	6.8 g/ 100 mL	25 mL
Instructions: Mix in 40 mL water. Adjust the pH to 8.0 using NaOH and then add water to a total volume of 50 mL. Always prepare fresh.					

5X SDS-PAGE Buffer, 10 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
Tris-HCl, pH 6.8–7.0	300mM	121.14	1 M	121.14 g/ 1 L	3 mL
Glycerol	50% (v/v)	–	100% (v/v)	–	5 mL
SDS	5% (w/v)	–	–	–	0.5 g
Bromophenol blue	0.05% (w/v)	–	4%	–	125 µL
DTT	250 mM	154.25	1 M	1.54 g/ 10 mL	125 µL/aliquot
Instructions: Make sure to prepare a 1 M Tris-HCl stock by dissolving Tris base in 500 mL deionized water, adding HCl to a pH of 6.8–7.0, and adding water to a final volume of 1 L. For the SDS-PAGE Buffer, mix all components listed **except DTT** and add water to a total of 10 mL. Freeze 20 aliquots (375 µL each) at –20°C. Before use, add DTT to the needed single aliquots.					

Table 1. Magnetic bead suspension volumes suitable for given protein expression levels

Protein expression level	Amount of His-tagged protein per 1 mL culture	Amount His-tagged protein per 10 mL* culture	Volume 25% magnetic bead suspension per 10 mL culture	Minimum elution volume per 10 mL culture
<0.5 mg/L	<0.5 µg	<5 µg	2 µL	25 µL
1 mg/L	1 µg	10 µg	4 µL	25 µL
5 mg/L	5 µg	50 µg	20 µL	50 µL
10 mg/L	10 µg	100 µg	40 µL	100 µL
50 mg/L	50 µg	500 µg	200 µL	500 µL

* Volumes can be linearly scaled up or down for smaller or larger culture volumes.

Procedure

1. Thaw the *E. coli* cell pellet on ice.
2. Resuspend the cell pellet in 1 mL Lysis Buffer supplemented with 1 mg/mL lysozyme.
3. Add 6 U Benzonase® (3 units/mL bacterial culture) to the lysate to reduce viscosity caused by genomic DNA.
4. Incubate for 30 min on ice, if necessary. Otherwise, incubating at room temperature (20–25°C) may be more efficient.
5. Centrifuge the lysate for 30 min at 10,000xg and 4°C. Collect the supernatant.
6. Resuspend the Cambio His Affinity MagBeads by vortexing. Transfer 40 µL of the 25% magnetic bead suspension into a conical microcentrifuge tube (or the volume adjusted to the expression level; see Table 1).
7. Add 500 µL Lysis Buffer and mix by vortexing. Place the tube on a magnetic microtube stand until the beads are separated and discard the supernatant.
8. Pipet 1 mL of the cleared lysate onto the equilibrated magnetic beads, and incubate the lysate-magnetic bead mixture at 4°C for 1 h on an end-over-end shaker.
9. Place the tube on the magnetic microtube stand until the beads separate and remove the supernatant.
10. Remove the tube from the magnet. Add 500 µL Wash Buffer and mix by vortexing. Place the tube again on the magnetic microtube stand and allow the beads to separate. Remove the supernatant.
11. Repeat step 10 twice.
12. Elute the His-tagged protein using 100 µL Elution Buffer (or the volume adjusted to the expression level; see Table 1).
13. Repeat step 12. Collect each elution fraction in a separate tube and determine the protein concentration of each fraction.
14. Analyze all fractions by SDS-PAGE.
15. Optional: Perform Western Blot experiment using PentaHis Antibody.

Optional: Freezing the cell pellet at −20°C for 30 min prior to incubation at room temperature improves lysis by lysozyme.

Optional: Add 1 tablet protease inhibitor cocktail to the Lysis Buffer.

Tip: Lysis Buffer contains 10 mM imidazole to prevent binding of untagged proteins. If His-tagged proteins do not bind under these conditions, reduce the imidazole concentration to 1–5 mM.

Note: The supernatant contains the **cleared lysate fraction.**
We recommend to take aliquots of all fractions for SDS-PAGE analysis.

This is the **flow-through fraction**.

These are the **wash fractions.**

These are the **elution fractions.**

Note: Do not boil membrane proteins. Instead, incubate the sample at 46°C for 30 min in preparation for SDS-PAGE analysis.

Trademarks: Benzonase®(Merck KGaA);
Novagen®(EMD Biosciences).