Cambio

Purification of His-tagged Proteins Under Denaturing 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 denaturing 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, cells are lysed with a high concentration of urea, which also aids to dissolve insoluble protein aggregates. The His-tagged protein is purified from the cleared lysate under denaturing conditions in a bind-wash-elute procedure. Binding occurs at slightly alkaline pH, while washing and elution are done with a stepwise pH decrease.

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).

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
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)
Urea
Sodium hydroxide (NaOH)
Dithiothreitol (DTT)
Glycerol
Sodium dodecyl sulfate (SDS)
Bromophenol blue
Tris base
Hydrochloric acid (HCl)
Optional: anti-His Antibody

Solutions and buffers

Denaturing Lysis Buffer, pH 8.0, 50 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
NaH_2PO_4	100 mM	119.98	0.5 M	29.99 g/ 500 mL	10 mL
Tris base	10 mM	121.14	1 M	12.11 g/ 100 mL	0.5 mL
Urea	8 M	60.06	–	–	24 g
Dissolve urea in 30 mL water and then add the remaining components. Adjust pH to 8.0 with HCl and add water to a total volume of 50 mL. Due to urea dissociation, adjust the pH immediately before use.					

Denaturing Wash Buffer, ph 6.3, 50 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
NaH_2PO_4	100 mM	119.98	0.5 M	29.99 g/ 500 mL	10 mL
Tris base	10 mM	121.14	1 M	12.11 g/ 100 mL	0.5 mL
Urea	8 M	60.06	–	–	24 g
Dissolve urea in 30 mL water and then add the remaining components. Adjust pH to 6.3 with HCl and add water to a total volume of 50 mL. Due to urea dissociation, adjust the pH immediately before use.					

Denaturing Elution Buffer, pH 4.5, 50 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
NaH_2PO_4	100 mM	119.98	0.5 M	29.99 g/ 500 mL	10 mL
Tris base	10 mM	121.14	1 M	12.11 g/ 500 mL	0.5 mL
Urea	8 M	60.06	–	–	24 g
Dissolve urea in 30 mL water and then add the remaining components. Adjust pH to 4.5 with HCl and add water to a total volume of 50 mL. Due to urea dissociation, adjust the pH immediately before use.					

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 Denaturing Lysis Buffer.
3. Incubate at room temperature for 30 min on an end-over-end shaker.
4. Centrifuge the lysate for 30 min at room temperature and 10,000 x g. Collect the supernatant.
5. Pipet 1 mL of the cleared lysate into a conical microcentrifuge tube.
6. Resuspend the Cambio His Affinity MagBeads by vortexing. Transfer 40 µL of the 25% magnetic beads suspension onto the lysate (or the volume adjusted to expression level; see Table 1).
7. Incubate the lysate-magnetic bead mixture at room temperature for 1 h on an end-over-end shaker.
8. Place the tube on the magnetic microtube stand until the beads separate and remove the supernatant.
9. Remove the tube from the magnet. Add 500 µL Denaturing Wash Buffer and mix by vortexing. Place the tube again on the magnetic microtube stand and allow the beads to separate. Remove the supernatant.
10. Repeat step 9 twice.
11. Elute the His-tagged protein using 100 µL Denaturing Elution Buffer (or the volume adjusted to the expression level; see Table 1).
12. Repeat step 11. Collect each elution fraction in a separate tube and determine the protein concentration of each fraction.
13. Analyze all fractions by SDS-PAGE.
14. Optional: Perform Western Blot experiment using PentaHis Antibody.

Optional: Benzonase® can be added to the lysate to reduce viscosity caused by nucleic acids (3 U/mL bacterial culture). **Read "about denaturation".** In addition, nucleic acids can be sheared by passing the lysate 10 times through a fine-gauge needle.

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

Tip: Briefly centrifuge the sample before placing it on the magnetic separator in order to collect liquid from the lid.

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

These are the **wash fractions.**

Tip: If the target protein is acid-labile, elution can be perfored with 250-500 mM imidazole.

These are the **elution fractions.**

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

About denaturation:

In some cases 8 M urea is not sufficient to completely solubilize inclusion bodies. In these cases the urea in the Denaturing Lysis Buffer can be replaced with 6 M guanidine hydrochloride (Gu-HCl). **Important:** Samples containing Gu-HCl cannot be directly applied to SDS-PAGE. Dilute the sample or subject it to a precipitation step (e.g., using trichloracetate (TCA) or similar) to remove the denaturant.

If using Benzonase to remove nucleic acids, the concentration of urea in the Denaturing Lysis Buffer must be decreased. Benzonase is active only at urea concentrations ≤7 M. In contrast, Gu-HCl inactivates Benzonase even at low concentrations.