TOYOBO

COSMO BIO CO., LTD.
Inspiration for Life Science

Instruction manual MagExtractor-His-tag-0905

F0987K

MagExtactor *-His-tag-*

NPK-701 100 preparations
Store at Store at 4°C

Contents

CAUTION
All reagents in this kit are intended for research purposes. Do not use for diagnostic or clinical purposes. Please observe general laboratory precaution and utilize safety while using this kit.

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[1] Introduction

Description

MagExtractor -*His-tag*- provides a simple and reliable method for the rapid purification of 6x histidine (His)-tagged proteins from bacterial lysate, utilizing magnetic nickel agarose beads. Sequential 6x histidine exhibits an affinity for metal ions, such as nickel (Ni^{2+}) [1]. This kit contains all components for purification.

Features

-Purification can be completed within 15 minutes minimum.
-This method is suitable for high-throughput purification using bacterial lysates containing cell debris, and utilizes magnetic separation as a purification principle.
-The binding capacity of the beads is 5-10 µg per 1 µl magnetic beads.

Notes

-This kit is suitable for purification from bacterial lysates, not for mammalian cell lysates. In the case of mammalian cell lysates, the purification specificity tends to be low.
-Yields of his-tagged proteins are dependent on protein expression levels and the location of tags.

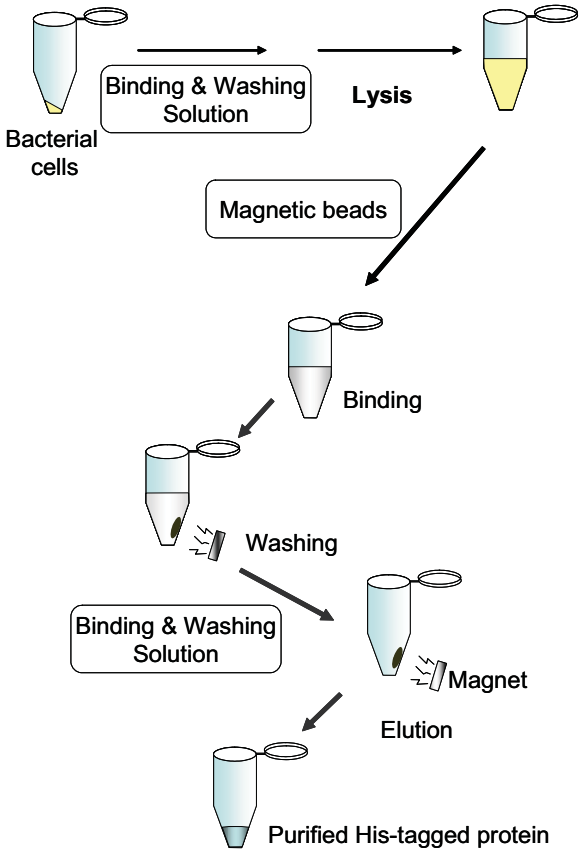

Fig. 2 Flow chart of purification

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[2] Components

This kit contains the following components for 100 preparations.

Binding & Washing Solution	176 ml	(Store at room temperature or 4°C)
Elution Solution	22 ml	(Store at room temperature or 4°C)
5 M NaCl	36 ml	(Store at room temperature or 4°C)
Magnetic Beads	4.4 ml	(Store at 4°C)

Caution

-Do not freeze the magnetic beads.
-In the denaturation condition protocol, this kit is not sufficient for 100 preparations.

Notes

-Binding and Washing Solution contains 100 mM NaCl. This solution is adjusted to pH 8.0.
-Elution Solution contains 200 mM imidazole. This solution is adjusted to pH 7.4.

[3] Materials required

The following materials are required for purification.

(1) Reagents

-10 mg/ml Lysozyme solution <optional>
Dissolve 10 mg lysozyme in 1 ml Binding & Washing Solution.

-10 U/µl DNase I <optional>
-1 M $MgCl_2$ <optional>

(2) Instruments

-Vortex mixer
-Tube rotator
-Magnetic stand
-Sonicator <optional>
-Tube mixer <optional>
-Incubator (37°C) <optional>

Fig.3 Magnetic stand
Magical Trapper (Code No.MGS-101)

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[4] Protocol

His-tagged proteins can be purified by various methods depending on protein status and future applications of purified proteins. If the recovered proteins are incursion bodies, the proteins should be purified in a denatured condition.

The following table lists the features of each purification method.

Purification condition	Pretreatment of cells		Purity & Yield		Remarks
	Lysis	DNase I treatment	Purity	Yield	
Native condition	Sonication	-	High	High	Suitable for soluble proteins
	Lysozyme treatment	-	Medium	Medium	Suitable for soluble proteins
		+	High	Medium	Suitable for nucleic acid-related proteins, due to low nucleic acid contamination.
Denatured condition	Sonication	-	High	High	Suitable for aggregated proteins
Binding by lysing	Lysozyme treatment	+/-	Low/ Medium	Medium	Suitable for hydrophobic proteins

1. Purification under native conditions

(1) Pretreatment of bacterial cells

In the case of comparatively stable his-tagged proteins, bacterial cells should be lysed by sonication prior to purification. Because nucleic acids and cell walls are degraded by sonication, nucleic acid contamination in the purified his-tagged protein solutions can be decreased.

Lysozyme treatment is suitable for high-throughput purification of many specimens. Because lysozyme-treated cell lysates tend to contain nucleic acids, the addition of DNase I in the lysis step decreases the level of nucleic acid contamination.

(A) Sonication

Bacterial cell pellet
↓
↓← 570 µl Binding & Washing Solution
↓← 30 µl 5M NaCl
↓
Sonication at 4°C
↓
Centrifuge at 12,000 rpm for 1 minute
↓
Supernatant

Notes

-Sonication conditions should be optimized by monitoring absorption of supernatants at 280nm.

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(B) Lysozyme treatment

Bacterial cell pellet
↓
Freezing and thaw
↓
↓ ← 270 µl Binding & Washing Solution
↓
Suspension
↓
↓← 30 µl 10mg/ml Lysozyme
↓
Mix well with tube mixer at room temperature (25°C) for 30 minutes.
↓
↓← 15 µl 5M NaCl
↓
Centrifuge at 12,000 rpm for 1 minute
↓
Supernatant

Notes

-For an effective lysozyme treatment, bacterial cells should be frozen and thawed prior to purification.
-Because NaCl inhibits lysozyme activity, NaCl should be added subsequent to lysozyme treatment.

(B) Lysozyme & DNase I treatment

Bacterial cell pellet
↓
Freezing and thaw
↓
↓ ← 270 µl Binding & Washing Solution
↓ ← 6 µl 1M $MgCl_2$
↓
Suspension
↓
↓← 30 µl 10 mg/ml Lysozyme
↓← 1 µl 10 U/µl DNase I
↓
Mix well with tube mixer at 37°C for 10 minutes.
↓
↓← 15 µl 5M NaCl
↓
Centrifuge at 12,000 rpm for 1 minute
↓
Supernatant

Notes

-For effective lysozyme treatment, bacterial cells should be frozen and thawed prior to purification.
-Because NaCl inhibits lysozyme activity, NaCl should be added subsequent to lysozyme treatment.
-DNase I will be removed by subsequent purification steps.

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(2) Purification

Fig. 3
Magnetic separation

(A) Supernatant from the pre-treated lysate

(B) **<Binding>** Add 40µl **Magnetic Beads** to supernatant from the pretreated lysate.

Notes
Resuspend the **Magnetic Beads** completely prior to use.

(C) <**Binding**> Mix with tube rotator at room temperature (25°C) for 10-30 minutes.

Notes
-The speed of the tube rotator should be adjusted so that the **Magnetic Beads** are effectively suspended.
-A tube rotator can be substituted for a tube mixer. When using a tube mixer, the speed should not be set too high, because high speeds could cause denaturation of his-tagged proteins.
-Typical soluble proteins can be absorbed within 10 minutes.

(D) Place the tube on the magnetic stand. The magnet will attract the **Magnetic Beads**, separating from the specimen solution.

(E) Following magnetic capture, carefully remove the supernatant.

(F) **<Washing>** Add 500 µl **Binding & Washing Solution** to the beads and vortex for 10 seconds.

(G) Place the tube on the magnetic stand and collect the beads.

(H) Following magnetic capture, carefully remove the supernatant.

(I) **<Washing>** Repeat (F) - (H)

(J) **<Elution>** Add 100-200 µl **Elution Solution**.

Notes
-The elution volume should be dependent on the amount of his-tagged proteins.

(K) Mix with tube rotator at room temperature (25°C) for 1-10 minutes.

Notes
-Typical soluble proteins can be eluted within 1 minute.

(L) Place the tube on the magnetic stand after brief centrifugation.

(M) Collect the supernatant.

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2. Purification under denatured conditions

Aggregated proteins can be effectively purified under denatured conditions, although not all purified proteins will be refolded and reactivated. In this method, the Binding & Washing solution and Elution Solution might be insufficient for 100 preparations.

(1) Reagents required

(A) Binding & Washing Solution for denaturation

Add 0.57 g guanidine hydrochloride (or 0.48 g urea) to 1ml of Binding & Washing Solution and adjust pH to 8.0 with 1N HCl. The final concentrations of guanidine hydrochloride and urea are 6 M and 8 M, respectively.

(B) Elution Solution for denaturation

Add 0.57 g guanidine hydrochloride (or 0.48 g urea) to 1 ml of Elution Solution and adjust pH to 7.4 with 1N HCl. The final concentrations of guanidine hydrochloride and urea are 6 M and 8 M, respectively.

Caution

-Guanidine hydrochloride and urea are irritants. Take appropriate laboratory safety measures and wear gloves when handling.
-In this method, Binding & Washing solution and Elution Solution might not be sufficient for 100 preparations. Prepare the minimal volume for these reagents.

Notes

-Because the pH of these reagents is unstable, please use within 2 weeks after preparation.

(2) Pretreatment of bacterial cells

Bacterial cell pellet
↓
↓← 570 µl **Binding & Washing Solution for denaturation**
↓← 30 µl **5 M NaCl**
↓
Sonication at 4°C
↓
Centrifuge at 12,000 rpm for 1 minute
↓
Supernatant

Notes

-The sonication condition should be optimized as previously described.

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(2) Purification

(A) Supernatant from the pretreated lysate

(B) **<Binding>** Add 40µl **Magnetic Beads** to the supernatant from the pretreated lysate.

Notes
Resuspend the **Magnetic Beads** completely prior to use.

(C) <**Binding**> Mix with tube rotator at room temperature (25°C) for 10-30 minutes.

Notes
-The speed of the tube rotator should be adjusted so that the **Magnetic Beads** are effectively suspended. A tube rotator can be substituted for a tube mixer. When using a tube mixer, the speed should not be set too high, because high speeds could cause denaturation of his-tagged proteins.
-Typical soluble proteins can be absorbed within 10 minutes.

(D) Place the tube on the magnetic stand. The magnet will attract the **Magnetic Beads**, separating from the specimen solution.

(E) Following magnetic capture, carefully remove the supernatant .

(F) **<Washing>** Add 500 µl **Binding & Washing Solution for denaturation** to the beads and vortex for 10 seconds.

(G) Place the tube on the magnetic stand and collect the beads.

(H) Following magnetic capture, carefully remove the supernatant.

(I) **<Washing>** Repeat (G) - (I)

(J) **<Elution>** Add 100-200 µl **Elution Solution for denaturation**.

Notes
-The elution volume should be dependent on the amount of his-tagged proteins.

(K) Mix with tube rotator at room temperature (25°C) for 1-10 minutes.

Notes
-Typical soluble proteins can be eluted within 1 minute.

(L) Place the tube on the magnetic stand after brief centrifugation.

(M) Collect the supernatant.

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3. Purification with lysing (Simultaneous protocol)

This protocol is effective for purification of proteins. In addition, the method exhibits an affinity to cell debris and is suitable for high-throughput purification, although purified protein solutions tend to contain nucleic acids. Adding DNase I at the binding step can decrease the risk of nucleic acid contamination.

(1) Simultaneous protocol without DNase I treatment

(A) Freeze and thaw the cell pellet

(B) Add 230 µl Binding & Washing Solution and vortex well

(C) Add 30 µl 10 mg/ml Lysozyme

(D) Add 40 µl **Magnetic Beads** to the supernatant from the pretreated lysate.

Notes
Suspend the **Magnetic Beads** completely prior to use.

(E) <**Binding**> Mix with tube rotator at room temperature (25°C) for 30 minutes.

Notes
-The speed of the tube rotator should be adjusted so that **Magnetic Beads** are effectively suspended. A tube rotator can be substitute for a tube mixer. When using a tube mixer, the speed should not be set too high, because high speeds could cause denaturation of his-tagged proteins.

(E) Place the tube on the magnetic stand. The magnet will attract the **Magnetic Beads**, separating from the specimen solution.

(G) Following magnetic capture, carefully remove the supernatant.

(H) **<Washing>** Add 500 µl **Binding & Washing Solution** to the beads and vortex for 10 seconds.

(I) Place the tube on the magnetic stand and collect the beads.

(J) Following magnetic capture, carefully remove the supernatant.

(K) **<Washing>** Repeat (H) - (J)

(L) **<Elution>** Add 100-200 µl **Elution Solution**.

Notes
-The elution volume should be dependent on the amount of his-tagged proteins.

(M) Mix with tube rotator at room temperature (25°C) for 1-10 minutes.

(N) Place the tube on the magnetic stand after brief centrifugation.

(O) Collect the supernatant.

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(2) Simultaneous protocol with DNase I treatment

(A) Freeze and thaw the cell pellet

(B) Add 230 µl Binding & Washing Solution

(C) Add 6 µl 1 M $MgCl_2$ and vortex well

(C) Add 30 µl 10 mg/µl Lysozyme

(D) Add 1 µl 10 U/ml DNase I

(E) Add 40µl **Magnetic Beads** to the supernatant from the pretreated lysate.

Notes
Resuspend the **Magnetic Beads** completely prior to use.

(F) <**Binding**> Mix with tube rotator at room temperature (37°C) for 10 minutes.

Notes
-The speed of the tube rotator should be adjusted so that **Magnetic Beads** are effectively suspended. A tube rotator can be substituted for a tube mixer. When using a tube mixer, the speed should not be set too high, because high speeds could cause denaturation of his-tagged proteins.

(G) Place the tube on the magnetic stand. The magnet will attract the **Magnetic Beads**, separating from the specimen solution.

(H) Following magnetic capture, carefully remove the supernatant.

(I) **<Washing>** Add 500 µl **Binding & Washing Solution** to the beads and vortex for 10 seconds.

(J) Place the tubes on the magnetic stand and collect the beads.

(K) Following magnetic capture, carefully remove the supernatant.

(L) **<Washing>** Repeat (I) - (K)

(M) <**Elution**> Add 100-200 µl **Elution Solution**.

Notes
-The elution volume should be dependent on the amount of his-tagged proteins.

(N) Mix with tube rotator at room temperature (25°C) for 1-10 minutes.

(O) Place the tube on the magnetic stand after brief centrifugation.

(P) Collect the supernatant.

Notes

-The DNase I will be removed by subsequent purification steps.

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[5] Protein analysis

1. Measurement of protein concentration

Protein concentration should be measured by the following method. The method based on absorbance at 280 nm is not appropriate, due to DNA contamination in protein solutions.

<Bradford method>
M. M. Bradford et al., *Anal. Biochem.*, **72:** 248-254 (1976)

<Lowry method>
O. H. Lowry et al., *J. Biol. Chem.*, **193:** 265-275 (1951)

<BCA method>
P. K. Smith et al., Anal. Biochem., **150:** 76-85 (1985)

2. SDS-PAGE

Protein purified under denatured conditions can be applied to SDS-PAGE gel without heat denaturation. In this condition, an electrophoresis gel should be washed with a de-staining solution prior to staining.
If clear SDS-PAGE patterns are desired, protein specimens should be dialyzed with low salt solutions.

3. Replacement of buffer

Buffer solutions containing purified proteins should be replaced by a low salt solution using typical methods (*e.g.,* dialysis or ultrafiltration).
Purified protein refolding under denatured conditions should be performed by dialysis method using serially diluted denatured solution (*i.e.,* guanidine hydrochloride or urea).

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[6] Troubleshooting

Symptom	Cause	Solution
No binding of his-tagged proteins	Insufficient cell lysis	Lysis conditions should be optimized by monitoring absorption of supernatants at 280 nm.
	Low protein expression	Reconfirm expression plasmids.
	Steric exclusion	-Alter position of the tag -Purify under denatured conditions
	Unexpected removal of the tag	-Check the protease site between the protein and tag. -Use a protease-deficient cell line for expression.
	Poor absorption conditions	-The pH of denaturing buffers should be checked prior to use. Denaturing buffers are easily altered. -The binding buffer should not contain chelaters or reductants.
His-tagged proteins in non-absorbed or washing fractions.	Excess cells	-Increase the amount of Magnetic Beads. -Decrease the number of cells.
No elution of his-tagged proteins	Strong binding of proteins to beads	-Add detergents up to 1.0% of Elution Solution (*e.g.,* Nonidet P-40, Triton X-100, or Tween 20). -Elute with Elution Buffer containing ≤ 100 mM EDTA.
Low purity	Low washing stringency	-Wash beads with Binding & Washing Solution containing ≤ 1 M NaCl. -Wash beads with Binding & Washing Solution containing ≤ 1% detergents (*e.g.,* Nonidet P-40, Triton X-100, or Tween 20). -Wash beads with Binding & Washing Solution containing 5-10% (v/v) Elution Solution. The solution should be 10-20 mM. -Increase the number of washing steps.
	Stringent elution conditions	-Decrease the magnitude of vortexing when eluting. -Decrease the elution time.
	Low protein expression	-Decrease the amount of Magnetic Beads by 4 µl-increments.
	Excess cells	-Decrease the number of cells by 10% increments. -Increase the number of washing steps. -Repurify the recovered solution after replacing the buffer.
	Non-specific binding to his-tagged proteins	-Wash beads with Binding & Washing Solution containing ≤ 1 M NaCl. -Wash beads with Binding & Washing Solution containing ≤ 1% detergents (e.g., Nonidet P-40, Triton X-100, or Tween 20).

[7] References

1) J. Schmitt, H. Hess and H.G.Stunnenberg, *Molecular Biology Reports*., 18: 223 (1993)

[8] Related products

Product name	Package	Code No.
Magnetic stand **Mag*ical Trapper***	1 pieces	MGS-101

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