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Rapid Purification of His-Fusion Proteins Kit

Introduction

The Rapid Purification of His-Fusion Proteins kit is designed for rapid, single-step purification of recombinant His-tagged fusion proteins from bacteria or other organisms based on magnetic beads.

Components

Contents \ Cat. No.	MH 101	MH 102	MH 103	MH 104	MH 105	MH 106
BcMag. His Beads	1.0ml	2.0ml	5.0ml	2.0ml	5.0ml	10ml
5x Binding /Washing Buffer I	12ml	24ml	60ml	X	X	X
1x Elution Buffer I	6.0ml	12ml	30ml	X	X	X

Buffer Composition

- BcMag.His Beads (suspended in 1% $NiSO_4.6H_2O$)
- 1x Binding/Washing Buffer I (0.5 M NaCl, 100 mM HEPES, 10 mM imidazole, pH 8.0)
- 1x Elution Buffer I (100 mM HEPES,0.5 M imidazole, pH 8.0)
- PBS Buffer (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.5)

Binding capacity: ~ 15-20 mg His-tagged fusion protein / ml magnetic beads

Storage: 4° C (Do not freeze)

Protocol

Designing a universal protocol for purification of DNA or RNA is relatively straightforward because of nucleic acids relatively uniform biochemical properties. However it is very difficult to design a universal kit for protein purification because each protein has a different composition and structure. In order to get the best results, each user must determine the optimal working conditions for purification of individual fusion proteins based on the suggestions described in the troubleshooting section.

Note: Prior to purifying the His-Tagged fusion protein, you should equilibrate all the reagents contained in the kit at room temperature and make 1x working solutions by diluting 5x stock solutions with 4 volumes of double distilled H_2O.

A. Cell Extract Preparation

1. Harvest cells by centrifugation at 10,000 xg for 6 min, remove the supernat completely.

2. Freeze cell pellet at -80° C for 1 hour.

3. Thaw cell pellet on ice and re-suspend cells with 3 ml 1x Binding/Washing Buffer I per 50 ml culture.

4. Break cells by brief pulses of sonication on ice until the sample is no longer viscous. Avoid sample heating. If desired, appropriate additives, such as non-ionic detergents (NP-40) or protease inhibitors (PMSF) can be added. However, DTT or 2-Mercaptoehanol can not added.

5. Separate soluble protein from insoluble protein by centrifugation at 10,000 xg for 6 min, carefully transfer the supernatant to a clean and pre-chilled tube and re-suspend pellet with 3 ml 1x Binding/Wshing Buffer I per 50 ml culture.

6. Aspirate 10 µl sample from soluble and insoluble fraction, add equal volume of 2x SDS sample loading buffer, boil for 5 min and run SDS-PAGE to determine the amount and solubility of the fusion protein

Note: If the target fusion protein forms an inclusion body(insoluble protein), it must be purified under denaturing conditions as described in Section C.

B. Purification of recombinant His-Tagged fusion protein under native conditions

1. Gently shake the bottle containing BcMag His Beads until the magnetic beads are completely suspended and transfer the appropriate amount of beads to a fresh tube.

Note: The optimal amount of beads to be used for each purification should be empirically determined by the user based on the amount of the His-Tagged fusion protein in crude sample. Too many magnetic beads will cause higher backgrounds; too little will cause lower yields. We recommend starting with 100 µl of the completely suspended beads per 1.5-2 mg of recombinant His-Tagged fusion protein.

2. Place the tube in a magnetic separator and wait for 2-3 min until supernatant becomes clear. Aspirate the supernatant completely, remove tube from the magnetic separator and re-suspend the beads with 4 volumes of 1x Binding/Washing Buffer I.

Note: Magnetic separators are commercially available from Bioclone Inc.: BcMag separator-2 for holding two individual 1.5 ml centrifuge tube, Cat. No. MS-01; BcMag separator-6 for holding six individual 1.5 ml centrifuge tubes, Cat. No.MS-02; BcMag separator-24 for holding twenty-four individual 1.5 ml centrifuge tubes, Cat. No. MS-03; BcMag separator-50 for holding one 50 ml and one 15 ml centrifuge tube, Cat. No. MS-04.

3. Repeat (step 2) one time.

4. Place the tube in the magnetic separator and discard the supernatant. Resuspend the beads with one volume of 1x Binding/ Washing Buffer I.

5. Combine the prepared cell extract with the magnetic beads, mix well by inverting several times and incubate for 5 min with rotational mixing.

6. Place the tube in the magnetic separator, save small portions of the supernatant and discard the rest.

Note: Save an aliquot of the supernatant for the further analysis since some protein might not bind to the magnetic beads.

7. Wash the magnetic beads by adding 8 volumes of 1x Binding/ Washing Buffer I and re-suspend the beads by pipetting several times, place the tube in the magnetic separator again and remove the supernatant.

8. Repeat (step 7) two times.

9. Remove the tube from the magnetic separator and elute the bound protein from the magnetic beads by adding desired volumes of 1x Elution Buffer I. Resuspend the beads very well by pipetting several times and incubate at room temperature for 5 min with rotational mixing. Place the tube in the magnetic separator and carefully transfer the supernatant to a clean tube.

10. Repeat (Step 9) one time.

11. Aspirate 10 - 20 µl of the eluted protein and supernatant form step 6 above, respectively, and analyze by running SDS-PAGE to confirm the presence of the target protein.

12. Free imidazole may be removed from the sample by dialysis at 4° C against a buffer of choice.

C. Purification of recombinant His-Tagged fusion protein under denaturing conditions

Buffer preparation:

- 1x Binding/Washing Buffer II: 1x Binding/Washing Buffer I + 6.0 M Urea
- 1x Elution Buffer II: 1x Elution Buffer I + 6.0 M Urea

Purification of His-Tagged fusion protein:Purification of His-Tagged fusion protein:

1. Harvest cells by centrifugation at 10,000 xg for 10 min, remove all the supernatant and re-suspend cell pellet in 3 ml ice-cold PBS Buffer per 50ml culture.

2. Freeze cell pellet at -80^{o} C for 1 hour.

3. Thaw cell pellet in a tube on ice and re-suspend cells in 3 ml ice-cold 1x Binding/washing I Buffer per 50 ml culture.

4. Break cells by brief pulses of sonication on ice until the sample is no longer viscous. Avoid sample heating.

5. Purify inclusion bodies by centrifugation at 12,000 xg for 10 min, remove all the supernatant and re-suspend the pellet in 3 ml ice-cold 1x Binding/Washing Buffer II per 50 ml culture.

6. Incubate for 10-30 min with rotational mixing at room temperature to completely dissolve the inclusion bodies.

8. Centrifuge at 12,000 xg for 10 min and transfer all the supernatant to a fresh tube.

9. Continue the same protein purification procedure as B section except for using 1x Binding/Washing Buffer II and 1x Elution Buffer II.

Troubleshooting

Problem: The yield of the purified fusion protein is too low or undetectable in eluted protein solution by SDS-PAGE.

Possible causes:

1. The reading frame is wrong.

Suggestion:

- Confirm sequence to ensure that the reading frame is correct.

2. The His-tag is not accessible due to protein conformation.

Suggestion:

- Purify the fusion protein under denaturing condition.
- Reclone and move tag to the other end of protein.

3. The fusion protein is unstable.

Suggestion:

- Add protease inhibitor to Binding/Washing Buffer

4. The fusion protein does not bind to the BcMag .His beads.

Suggestion:

- Check the pH of all the buffers and solutions.

5. The fusion protein is not efficiently eluted from BcMag .His beads.

Suggestion:

- Increase the concentration of imidazole to 0.5-1.0 M in the Elution Buffer.- Use 0.5-1.0 M sodium citrate (pH 4.0) to elute protein. After elution, immediately adjust pH to 7.0 by using 1.0 M Tris-HCl (pH 9.0).
- Use 100 mM EDTA (pH 8.0) to elute the protein.

6. Fusion protein appears in the Washing Buffer.

Suggestion:

- Reduce the concentration of imidazole.
- Increase pH slightly in Binding/Washing Buffer.

7. BcMag.His beads used is insufficient for purification.

Suggestion:

- Increase amount of BcMag.His beads.

Problem: Multiple bands observed in the eluted protein.

Possible cause:

1. Degradation of the fusion protein

Suggestion:

- Add appropriate protease inhibitor
- Use protease-deficient expression host

2. Some host proteins may interact with fusion protein.

Suggestion:

- Add NaCl (0.5-1.0 M, final concentration)in the Binding and Elution Buffers.

3. Over-sonication will cause binding of some proteins to the fusion protein.

Suggestion:

- Use milder sonication conditions or other lysis method, such as lysozyme.

4. Sample is too viscous.

Suggestion:

- Use syringe to shear the genomic DNA contained in the sample.
- Dilute the sample with Binding/Washing Buffer.

5. Host contains histidine-rich proteins.

Suggestion:

- Try to use second purification techniques such as ion exchange chromatography.

6. Incomplete washing

Suggestion:

- Increase washing time and washing volume

Problem: Eluted proteins lose activity.

Possible cause:

1. Some protein will lose or decrease activity when fused to his-tag.

Suggestion:

- Try to fuse his-tag at the other end of the protein
- Switch to other fusion tags, such as GST

2. The Elution Buffer is too harsh for protein.

Suggestion:

- Try to use other elution buffer as described above.