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Rapid Purification of GST-Fusion Proteins Kit

Introduction

The Rapid Purification of GST-Fusion Proteins kit is designed for rapid, single-step purification of recombinant glutathione S-transferase (GST) fusion proteins from bacteria or other organisms based on magnetic beads.

Components

Contents \ Cat. No.	MG 101	MG 102	MG 103	MG 104	MG 105	MG 106
BcMag.GST Beads	1.0ml	2.0ml	2.0ml	5.0ml	10ml	50ml
10x GST Binding/ Washing buffer	12 ml	24 ml	X	X	X	X
2x GST Elution Buffer	5 ml	10 ml	X	X	X	X
Glutathione (Reduced)	0.15 g	0.3 g	X	X	X	X

Buffer Composition

- BcMag.GST Beads (Suspended in 0.05 M Na_2HPO_4, pH 7.5, 0.15 M NaCl, 0.01% NaN_3)
- 1x GST Binding/Washing Buffer (0.14 M NaCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 2.7 mM KCl, pH 7.5)
- 1x GST Elution Buffer (50 m M Tris-HCl, pH 8.0)
  Note: Dissolve 100 mg Glutathione (reduced) in 10 ml of 1x Elution Buffer. Prepare fresh.
- 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: ~10 mg GST-Tag fusion protein / ml magnetic bead

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 GST fusion proteins, you should equilibrate all the reagents contained in the kit to room temperature.

A. Cell Extract Preparation

1. Harvest cells by centrifugation at 10,000 xg for 10 min, remove the entire supernatant and re-suspend cell pellet in 3 ml ice-cold PBS Buffer per 50 ml culture.
2. Concentrate cells by centrifugation at 10,000 xg for 10 min and remove all the supernatant. Freeze cell pellet at - 80° C for 1 hr.
3. Thaw cell pellet on ice and re-suspend cells in 3 ml ice-cold 1x Binding/Washing 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. If desired, appropriate additives, such as non-ionic detergents (NP-40) or protease inhibitors (PMSF), can be added.

Note: The binding of GST to BcMag.GST Beads is unaffected by 1% Triton X 100, 1% Tween 20, 1% CTAB, 10 mM DTT, 0.03% SDS, or 0.1% NP-40. These chemicals may reduce non-specific binding.

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 in 3 ml ice-cold 1x Binding/Washing Buffer per 50 ml culture.
6. Aspirate 10 µl sample from soluble and insoluble fractions, add equal volume of 2x SDS sample Loading Buffer, boil for 5 min and run SDS-PAGE to determine the solubility of the target GST fusion protein.

Note: If the target GST fusion protein forms an inclusion body (insoluble protein), the inclusion body has to be properly solubilized and refolded prior to purification.

B. Purification of recombinant GST fusion protein

1. Gently shake the bottle of BcMag.GST 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 GST 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 1.0 mg of active recombinant GST fusion protein. Because only the active GST fusion protein can bind to the magnetic beads, the level of active GST fusion protein in crude or purified samples should be assayed with appropriate means, such as kits available from Novagen.

2. Place the tube in a magnetic separator and wait for 1-3 min until supernatant becomes clear. Remove and discard all the supernatant, romove the tube from the magnetic separator and re-suspend the beads with 4 volumes of 1x Binding/Washing Buffer by gently pipetting several times.

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 GST Binding/Washing Buffer.
5. Combine the prepared cell extract with the magnetic beads, mix well by gently pipetting several times and incubate at room temperature for 15 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 inactive GST fusion protein does not bind to the magnetic beads.

7. Wash the magnetic beads by adding 8 volumes of 1x GST Binding/Washing Buffer and re-suspend the beads, place the tube in the magnetic separator again and remove the supernatant.
8. Repeat (step 7) three times
9. Remove the tube from the magnetic separator and elute the bound protein from the magnetic beads by adding desired volumes of 1x GST Elution Buffer. Resuspend the beads very well and incubate at room temperature for 15 min with rotational mixing.

Place the tube in the magnetic separator and carefully transfer the supernatant to a clean tube.

10. Repeat (Step 9) once or until the absorbance of elute at 280 nm approaches background level (OD 280 < 0.05).

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

Note: If the target GST fusion protein does not have functional GST, user has to switch to other alternative purification methods.

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

C. BcMag.GST beads reuse and storage

If the target GST fusion protein is the same, BcMag.GST beads can be reused three times without regeneration. However, if target GST fusion is different or the magnetic beads binding capacity declines, the BcMag.GST beads must be regenerated based on the following protocol:

1. Wash the magnetic beads with 10 volumes of regeneration buffer I (50 mM Tris-HCl, pH 8.0, 0.5 M NaCl) by use of the magnetic separator described above.

2. Wash the magnetic beads with 10 volumes of regeneration II (100 mM sodium acetate, pH 4.5, 0.5 M NaCl) by use of the magnetic separator.

3. Quickly equilibrate the magnetic beads by adding 10 volumes of 1x GST Binding/Washing Buffer. For long-term storage, the magnetic beads should be stored in 20% ethanol at 4° C.

D. Troubleshooting

Problem: The yield of the purified fusion protein is low or undetectable.

Possible cause:

1. The fusion protein forms an inclusion body.

Suggestion:

- Grow bacteria at 28-30° C.
- Reduce final concentration of IPTG to 0.1mM for protein induction.
- Reduce the induction time.
- Properly refold the inclusion body prior to the purification.

2. The fusion protein does not contain active GST.

Suggestion:

- Try other fusion protein methods, such as His-Tag to produce an alternative fusion protein.

3. Harsh sonication condition denatures the fusion protein

Suggestion:

- Try to use mild sonication condition or another method, such as lysozyme.

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

Suggestion:

- Add 5 mM DTT to a final concentration in Binding Buffer prior to cell lysis.
- Check the pH of the Binding Buffer (pH should be 6.5-8.0).

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

Suggestion:

- Increase elution time.
- Increase the concentration of glutathione to 15 mM or higher in the Elution Buffer. (Please check final pH and adjust if necessary
- Adjust the pH of the Elution Buffer to 8.0-9.0 without increasing the glutathione concentration.
- Add Triton X-100 (0.1%, final concentration) or N-octylglucoside (2%, final concentration) or NaCl (0.1 - 0.2 M, final concentration) to the Elution Buffer.

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, such as chaperonins, may interact with fusion protein.

- Add DTT (5 mM, final concentration) in the Elution Buffer.
- Incubate the recombinant protein solution in Chaperonin Buffer (2 mM ATP, 10 mM $MgSO_4$, 50 mM Tris-HCl) at 37° C for 10 min prior to the purification.

3. Over-sonication will cause some protein to bind to the fusion protein.

- Use milder sonication condition or another lysis method.