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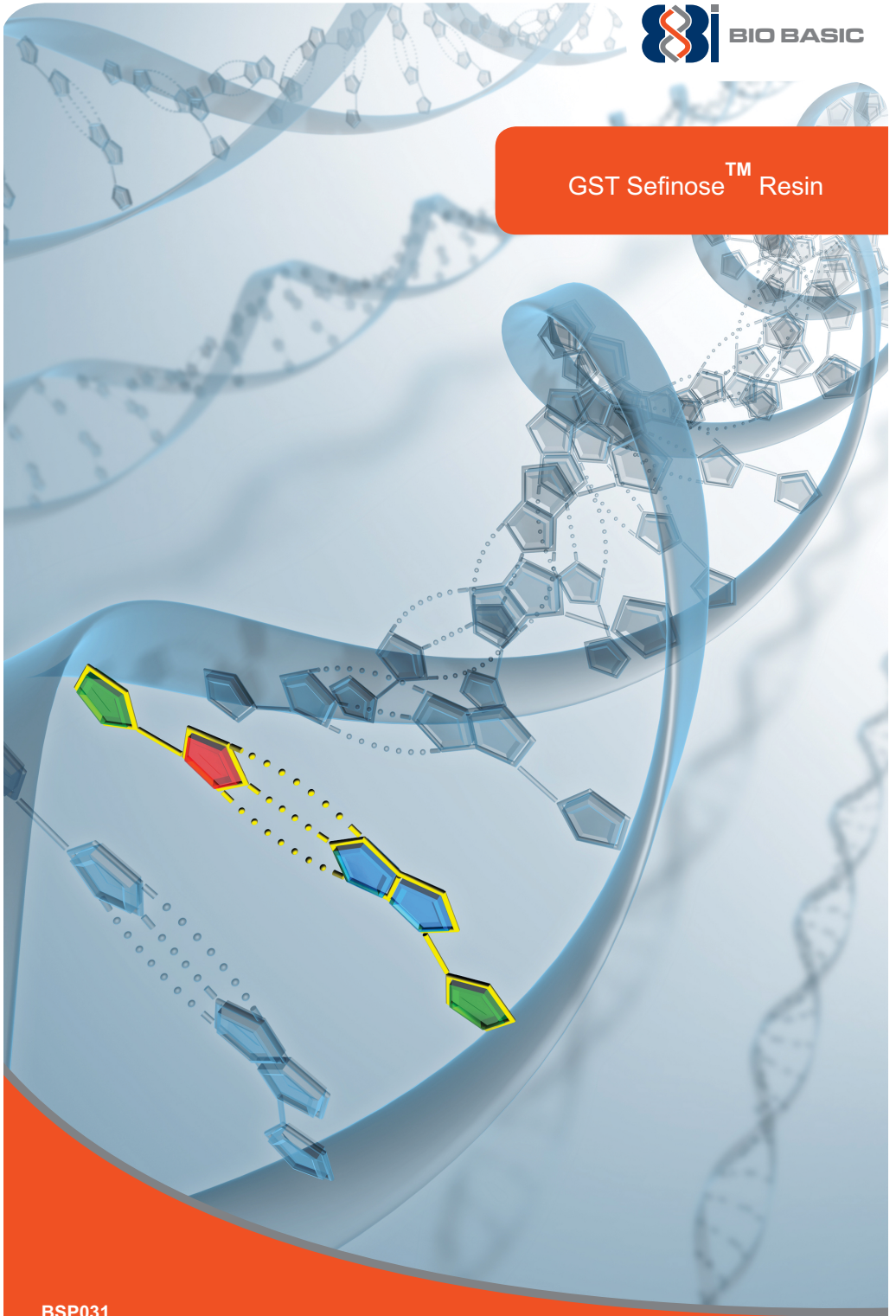
GST Sefinose™ Resin



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For Research Use Only

GST Sefinose™ Resin
SKU: BSP031

Description

The glutathione ligand is coupled via a 10-carbon linker to highly cross-linked 6% agarose. The coupling of Glutathione Sefinose™ resin is optimized to provide high binding capacity and one-step purification of glutathione S-transferase (GST) tagged proteins expressed from pGEX series vectors, as well as other glutathione S-transferases and glutathione binding proteins. GST-tagged proteins can be purified directly from pre-treated bacterial lysates using Glutathione Sefinose resin. The tagged proteins are eluted under mild, non-denaturing conditions that preserve protein antigenicity and function. The Glutathione sefinose resin is an excellent choice for high performance purifications. Glutathione sefinose resin is also available in conveniently pre-packed 1 ml, 5 ml and 10 ml column sizes.

If removal of the GST moiety (a naturally occurring protein with Mr 26 000) is required, the tagged protein can be digested with appropriate site-specific protease while bound to Glutathione Sefinose or, alternatively after elution. Cleavage of GST-tagged protein bound to resin eliminates the extra step of separating the released protein from GST. The cleaved target protein is eluted using binding buffer.

Multiple bands are observed after electrophoresis analysis of cleaved target protein:

A) Determine when the bands appear

Test to be certain that additional bands are not present prior to thrombin or factor Xa cleavage. Such bands may be the result of proteolysis in the host bacteria.

B) Tagged partner may contain recognition sequences for PreScission Protease, thrombin or factor Xa

Check the sequences.

Incomplete cleavage of GST-tagged proteins

A) Incomplete cleavage of GST-tagged proteins

Thrombin, at least 10 units/mg tagged protein. One cleavage unit of thrombin digests ⩾ 90 % of 100 µg of a test tagged protein in 16 hours at +22°C. Factor Xa, at least 1% (w/w) tagged protein. For some tagged proteins, up to 5% factor Xa can be used. The optimum amount must be determined empirically. In some cases, a tagged protein concentration of 1 mg/ml has been found to give optimal results. Adding ≈ 0.5% SDS (w/v) to the reaction buffer can significantly improve factor Xa cleavage with some tagged proteins. Various concentrations of SDS should be tested to find the optimum concentration.

B) Increase incubation time and enzyme concentration

For thrombin or factor Xa, increase the reaction time to 20 hours or more if the tagged protein is not degraded by extensive incubation. The amount of enzymes can also be increased

C) Verify the presence of specific cleavage sites

Check the DNA sequence of the construct. Compare it with a known sequence and verify that the different specific cleavage sites for the enzyme used have not been altered during the cloning of your tagged protein

D) Ensure that cleavage enzyme inhibitors are absent

Factor Xa: Buffer exchange on dialyse against 50 mM Tris-HCl, 150 mM NaCl, 1 mM $CaCl_2$, pH 7.4.

Factor Xa is not properly activated: Functional factor Xa requires activation of factor X with Russell's viper venom. Activation conditions are a ratio of Russell's viper venom to factor Xa of 1% in 8 mM Tris-HCl, 70 mM NaCl, 8 mM $CaCl_2$, pH 8.0. Incubate at +37°C for 5 minutes.

The first Amino acid after the factor Xa recognition sequence is Arg or Pro: Check the sequence of the tagged partner to be sure that the first three nucleotides after the factor Xa recognition sequence do not code for Arg or Pro.

Features

Average bead size	50 µm
Protein binding capacity*	Approx.5-10 mg GST-tagged protein/1ml resin
Max flow velocity	300 cm/h
Ligand density	10-20 µmol/ml GSH resin
pH stability, short-term (2 h)	3–12
Storage	20% ethanol
Storage temperature	+4 to +30ºC

NOTE: Binding of GST to glutathione is flow-rate dependent. The lower the flow rate, the higher the binding capacity. Flow rate is also essential during sample loading and elution steps.

Sample Preparation

The protocol below has been used successfully in many laboratories, but other established procedures may also work.

1. For protein expressed in E. coli. Dilute the cell paste: Add 5–10 ml of binding buffer for each gram of cell paste and mix.
2. Enzymatic lysis: Add to a final concentration of 0.2 mg/ml lysozyme, 20 µg/ml DNAse, 1 mM $MgCl_2$, 1 mM PMSF or other protease inhibitors. The inhibitors must not have any effect on binding ability of the resin. Stir for 30 minutes at 4°C.
3. Mechanical lysis (optional): Sonication, homogenization, repeated freeze/thaw or similar techniques.
4. Adjust the pH of the lysate to pH 7.4: Do not use strong bases or acids for pH-adjustment (with the risk of precipitation).
5. Centrifuge the lysate: Transfer clear lysate to a clean tube and centrifuge at 12000 rpm for 20 minutes at 4°C.
6. Collect supernatants and perform the purification.

NOTE: The sample should be centrifuged and/or filtered through a 0.45 µm filter immediately before it is applied to the resin. If the sample is too viscous, dilute it with binding buffer to prevent clogging the column.

7. As for the preparation of Insect and Mammalian Cell Lysate, please use other commercial Reagents or kits (such as BSP020 Insect Cell Protein Lysis Buffer, BSP022, Animal Cells Protein Lysis Buffer) or refer to relative documents.

Purification Procedures

Procedures for Purification of GST-Tagged Proteins by Batch Method

NOTE: Purification conditions can be scaled as needed. The procedure may be performed at room temperature or at 4ºC.

1. Add an appropriate amount of GST Sefinose™ Resin to a tube. Centrifuge tube for 2 minutes at 700 x g and carefully remove and discard the supernatant.
2. Add 10 resin-bed volumes of Binding/wash buffer and mix until the resin is fully suspended.
3. Centrifuge tube for 2 minutes at 700 x g and carefully remove and discard buffer.
4. As for Insect and Mammalian Cell Lysates, prepare sample by mixing the protein extract with 5 volumes of Binding/wash buffer. Other ratios may be used but need to be determined empirically.
5. Add the protein extract of E. coli Lysates or mixture of Insect and Mammalian Cell Lysates and Binding/wash buffer to the equilibrated resin and mix on an end-over-end rotator for 30-60 minutes at room temperature or 4°C.
6. Centrifuge the tube for 2 minutes at 700 x g. If desired, save supernatant for downstream analysis.
7. Wash the resin with 5-10 resin-bed volumes of Binding/wash buffer. Centrifuge the tube for 2 minutes at 700 g and remove supernatant. If desired, save supernatant for downstream analysis.
8. Repeat wash step and monitor supernatant by measuring its absorbance at 280 nm until baseline is reached.
9. Elute bound GST-tagged protein using one resin-bed volume of Elution Buffer. Centrifuge tube for 2 minutes at 700 x g. Carefully remove and save the supernatant. Repeat this step twice, saving each supernatant fraction in a separate tube.

B) More protease inhibitor required

Multiple bands may be a result of partial degradation of tagged proteins by proteases. Adding 1 mM PMSF to the lysis solution may improve results. A non-toxic, water-soluble alternative to PMSF is AEBSF.

NOTE: Serine protease inhibitors must be removed prior to cleavage by thrombin or factor Xa.

C) Use a protease-deficient host

Multiple bands may be the result of proteolysis in the host bacteria. If this is the case, the use of a host that is protease deficient may be required (e.g. lon-or ompT). E. coli BL21 is provided with the pGEX vectors. This strain is ompT.

D) Decrease sonication

Cell disruption is apparent by partial clearing of the suspension and can be checked by microscopic examination. Adding lysozyme (0.1 volume of a 10 mg/ml lysozyme solution in 25 mM Tris-HCl, pH 8.0) prior to sonication may improve results. Avoid frothing as this may denature the fusion protein. Over-sonication can also lead to the co-purification of host proteins with the GST-tagged protein.

E) Include an additional purification step

Additional bands may be caused by the co-purification of a variety of proteins known as chaperonins, which are involved in the correct folding of nascent proteins in E. coli. These include, but may not be limited to: DnaK (Mr~ 70 000), DnaJ (Mr~ 37 000), GrpE (Mr~ 40 000), GroEL (Mr~ 57 000) and GroES (Mr~ 10 000). Several methods for purifying GST-tagged proteins from these co-purifying proteins have been described.

F) Cross-adsorb antibody with E. coli proteins

Depending on the source of the anti-GST antibody, it may contain antibodies that react with various E. coli proteins that may be present in your tagged protein sample.

D) pH of the elution buffer

A low pH may limit elution from Glutathione Sefinose resin. Increasing the pH of the elution buffer to pH 8–9 may improve elution without requiring an increase in the concentration of glutathione used for elution.

E) Ionic strength of the elution buffer

Adding 0.1–0.2 M NaCl to the elution buffer may also improve results.

F) Reduced glutathione in elution is inactive

Use fresh elution buffer (reduced glutathione).

G) Add a non-ionic detergent to the elution buffer

Non-specific hydrophobic interactions may prevent solubilization and elution of tagged proteins from Glutathione Sefinose resin. Adding a non-ionic detergent may improve results. Adding 0.1% Triton X-100 or 2% N-octylglucoside can significantly improve elution of some GST-tagged proteins.

Multiple bands are observed after electrophoresis/Western Blotting analysis of eluted targeted protein

A) M 70 000 protein co-purifies with the GST-tagged protein

The Mr70 000 protein is probably a protein product of the E. coli gene dnaK. This protein is involved in protein folding in E. coli. It has been reported that this association can be disrupted by incubating the tagged protein in 50 mM Tris-HCl, 2 mM ATP, 10 mM $MgSO_4$, pH 7.4 for 10 minutes at +37°C prior to loading on Glutathione Sefinose resin. Alternatively, remove the DnaK protein by passing the tagged protein solution through ATP-agarose or by ion exchange.

10. Monitor protein elution by measuring the absorbance of the fractions at 280 nm. The eluted protein can be directly analyzed by SDS-PAGE.

Procedures for Purification of GST-tagged Proteins using a Gravity-flow Column

NOTE: Purification conditions can be scaled as desired. Perform the procedure at room temperature or at 4ºC.

1. Mix the slurry by gently inverting the bottle several times to completely suspend the resin. Use a pipette to transfer an appropriate volume of resin slurry to the Gravity-flow column. Allow the resin to settle and the storage buffer to drain from the column.
2. As for Insect and Mammalian Cell Lysates, prepare sample by mixing the protein extract with 5 volumes of Binding/wash. Other ratios may be used but need to be determined empirically.

   NOTE: For larger sample volumes, Do not exceed the column's binding capacity.
3. Equilibrate column with 10 resin-bed volumes of Binding/wash buffer. Using a flow rate of 0.5-1 ml/minute, all buffer to drain from resin or until A280 is stable.
4. Add the of E. coli Lysates or mixture of Insect and Mammalian Cell Lysates and Binding/wash buffer to the resin with a flow-rate of 0.5-1 ml/minute. Collect the flow-through in a tube for analysis. If desired, re-apply the flow-through once to maximize binding.
5. Wash resin with 5-10 resin-bed volumes of Binding/wash buffer and collect the flow-through. Repeat this step using a new collection tube until the absorbance of the flow-through fraction at 280 nm approaches baseline. Save the flow-through for measuring the binding efficiency to the medium, i.e. by SDS-PAGE.
6. Elute GST-tagged protein from the resin with two resin-bed volumes of Elution Buffer. Repeat this step twice, collecting each fraction in a separate tube.

7. Monitor protein elution by measuring the absorbance of the fractions at 280 nm The eluted protein can be directly analyzed by SDS-PAGE.

   NOTE: To remove glutathione for downstream applications use gel filtration (BSP089, Gravity column De-Salting).

Procedure for Restoring of GST Sefinose™ Resin

GST Sefinose™ Resin may be used at least five times without affecting protein yield or purity. Between each use, perform the procedure described below to remove residual glutathione and any nonspecifically adsorbed protein. To prevent cross-contamination of samples, designate a given column to one specific fusion protein.

1. Apply 5 resin-bed volumes of Regeneration Buffer 1(0.1M Tris containing 0.5M NaCl and 0.1% SDS, pH 8.5).
2. Apply 5 resin-bed volumes of ultrapure water.
3. Apply 5 resin-bed volumes of Regeneration Buffer 2(0.1M sodium acetate containing 0.5M NaCl and 0.1% SDS, pH 4.5).
4. Apply 5 resin-bed volumes of ultrapure water.
5. Wash the column with 5mL of 0.05% sodium azide (in water). Cap bottom and top of column. Store at 4 ºC.

Notes

1. One of the most important parameters affecting the binding of GST-tagged proteins or other glutathione binding proteins to Glutathione Sefinose is the flow rate. Due to the relatively slow binding kinetics between GST and glutathione, it is important to keep the flow rate low during sample application for maximum binding capacity. Protein characteristics, pH and temperature are other factors that may affect the binding capacity.
2. Volumes and times used for elution may vary among tagged proteins. Additional elutions with higher concentrations of glutathione may be required. Flow-through, wash and eluted material from the column should be monitored for GST-tagged proteins using SDS-PAGE in combination with Western Blot if necessary.

D) Glutathione Sefinose resin not equilibrated before use

Binding of GST-tagged proteins to Glutathione Sefinose resin is not efficient at pH less than 6.5 or greater than 8. Check that the Glutathione Sefinose resin has been equilibrated with a buffer 6.5 to 8.0 (e.g. PBS) before the clarified cell lysate is applied

E) Glutathione Sefinose resin too “old”

If the Glutathione Sefinose resin has already been used several times, it may be necessary to use fresh Glutathione sefinose resin.

F) Flow rate during sample load is too high

Decrease flow rate during sample load.

GST-tagged protein is not eluted efficiently from Glutathione Sefinose resin

A) Time of and flow rate of elution step

Decrease the flow during elution.

B) Volume of elution buffer

Sometimes, especially after on-column cleavage of tagged protein, larger volume of buffer may be necessary to elute the tagged protein.

C) Concentration of glutathione in the elution buffer

10 mM recommended in this protocol should be sufficient for most applications, but exceptions exist. Try 50 mM Tris- HCl, 20–40 mM educed glutathione, pH 8.0 as elution buffer.

2. Swirl gently to dissolve.

3. Freeze as 80 µl aliqouts and keep at –80 °C.

Steps are similar to those in Thrombin cleavage, however, one must wash the tagged protein bound Glutathione Sefinose resin with 10 bed volumes of factor Xa cleavage buffer or remove reduced glutathione from the eluate by dialysis against factor Xa cleavage buffer, before carry out the cleavage of fusion protein.

Troubleshooting

GST-tagged protein does not bind to Glutathione resin

A) GST-tagged protein denatured by sonication

Extensive sonication can denature the tagged protein and prevent it binding to Glutathione sefinose resin. Use mild sonication conditions during cell lysis. Conditions for sonication must be empirically determined.

B) No DTT prior to cell lysis and to buffers

Adding DTT to a final concentration of 1–10 mM may significantly increase binding of some GST-tagged proteins to Glutathione resin.

C) Parental pGEX and conformation change of fusion protein

Prepare a sonicate of cells harboring the parental pGEX plasmid and check binding to the matrix. If GST produced from the parental plasmid binds with high affinity, the fusion protein may have altered the conformation of GST, thereby reducing its affinity. Adequate results may be obtained by reducing the temperature used for binding to +4°C, and by limiting column washing.

3. The GST Detection Module can be used to optimize conditions for elution or to trace steps in the purification of a GST-tagged protein. The Module is designed to identify GST-tagged proteins using either a biochemical or an immunological assay.

4. The concentration of GST-tagged protein can be estimated by measuring the absorbance at 280 nm. The GST-tag can be approximated using the conversion; A280 ≈ 1 corresponds to ≈ 0.5 mg/ml.

5. The concentration of GST-tagged proteins may also be determined by standard chromogenic methods (e.g. Lowry, BCA, and Bradford assays). If Lowry or BCA assays are to be used, the sample must first be buffer exchanged using Desalting column or dialyze against PBS to remove glutathione, which can interfere with the protein measurement. The Bradford method can be used in the presence of glutathione.

6. The reuse of Glutathione Sefinose resin depends on the nature of the sample and should only be performed with identical samples to prevent cross-contamination.

7. If the sefinose resin appears to have lost binding capacity, it may be due to an accumulation of precipitate, denatured or nonspecifically bound proteins.

Cleavage of GST-Tagged Proteins

In most cases, the fusion partner of interest retains functional activity and the functional test can be performed using intact fusion with GST. If removal of the GST-tag is necessary, tagged proteins containing a thrombin recognition site or a factor Xa recognition site may be cleaved either while bound to Glutathione Sefinose or in solution after elution. Optimization of cleavage conditions is necessary. Samples can easily be removed at various time points and analyzed by SDS-PAGE to estimate the yield, purity and extent of digestion. The amount of protease used, the temperature and the length of incubation required for complete digestion may vary depending on the fusion protein. Optimal conditions for each tagged protein should be determined in pilot experiments.

Thrombin

Thrombin, Mr 37 000.
Thrombin cleavage buffer: PBS, pH 7.4.
Preparation of thrombin solution:

1. Dissolve 500 U thrombin in cold 500 µl PBS, pH 7.4 (1 U/µl).
2. Swirl gently to dissolve.
3. Freeze as 80 µl aliqouts and keep at –80°C.

Thrombin cleavage of GST-tagged protein bound to the resin

Assumption: 8 mg GST-tagged protein bound/ml resin

1. Follow steps A,B under ” Purification protein”, do not proceed with step C Elution step.
2. Prepare the thrombin mix: For each ml of Glutathione Sefinose bed volume, prepare a mixture of 80 µl (80 units) of thrombin and 920 µl of PBS, pH 7.4 (8 mg GST-tagged protein bound/ml resin).
3. Load the thrombin mix onto the column. Seal the column. If batch format is used, add the thrombin mixture to the Glutathion sefinose. Gently shake or rotate the resin.
4. Incubate at room temperature (22–25 °C) for 2-16 hours.
5. Following incubation, wash the column with approx. 3 bed volumes of PBS, pH 7.4. Collect the eluate in different tubes to avoid dilution of the tagged protein and analyze. If batch format is used, centrifuge the suspension at 500 x g for 5 minutes to pellet the Glutathione Sefinose and carefully transfer the eluate to a tube. The eluate will contain the protein of interest and thrombin, while the GST portion of the tagged protein will remain bound to the Glutathione sefinose resin.

Thrombin cleavage of eluted GST-tagged protein

Assumption: 8 mg GST-tagged protein bound/ml medium.

1. Add 10 µl (10 units) of thrombin solution for each mg of tagged protein in the eluate. If the amount of tagged protein in the eluate has not been determined, add 80 µl (80 U) of thrombin solution for each ml of Glutathione Sefinose resin from which the tagged protein was eluted. (8 mg tagged protein bound/ml resin).
2. Incubate at room temperature (22–25 °C) for 2–16 hours.
3. Once digestion is complete, GST can be removed by first removing glutathione by dialysis against PBS, pH 7.4. Follow this by applying the sample to washed and equilibrated Glutathione sefinose resin.
4. Incubate for 20–30 minutes at room temperature.
5. Sediment the medium by centrifugation at 500 × g for 5 minutes. The supernatant will contain the protein of interest and thrombin, while the GST portion of the tagged protein will remain bound to the Glutathione sefinose resin.

Factor Xa

Factor Xa, Mr 48 000.

NOTE: Factor Xa consists of two subunits linked by disulfide bridges. As glutathione can disrupt disulfide bridges, it should be removed from the sample prior to the cleavage reaction.
Factor Xa cleavage buffer: 50 mM Tris-HCl, 150 mM NaCl, 1 mM $CaCl_2$, pH 7.4.

Preparation of factor Xa solution:

1. Dissolve 400 U factor Xa in 400 µl cold water (1 U/µl).