GE Healthcare

GST GraviTrap

10 prepacked GST GraviTrap columns

Product booklet

Code: 28-9523-60

Page finder

1. Legal

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http//www.gelifesciences.com/sampleprep

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2. Handling

2.1. Safety warnings and precautions

Warning: For research use only.
Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle this product. Suitable protective clothing such as laboratory overalls, safety glasses, and gloves should be worn. Care should be taken to avoid contact with skin or eyes; if contact should occur, wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2. Storage

Store at 4°C to 30°C. Do not freeze.

3. Introduction

Prepacked GST GraviTrap™ contains Glutathione Sepharose™ 4B and is designed for single step, manual, gravity flow, purification of recombinant derivatives of glutathione S-transferase, other glutathione S-transferases or glutathione dependent proteins. GST-tagged proteins are recovered from the matrix under mild elution conditions (10 mM glutathione) which preserve functionality of the proteins.

The GST GraviTrap provides 10 prepacked Glutathione Sepharose 4B columns in a convenient gravity column format. Each gravity column contains 2 ml bed volume of Glutathione Sepharose 4B, enough to purify up to 20 mg of recombinant GST (rGST). The capacity will vary with the nature of the tagged protein and the exact binding conditions used.

For increased convenience a GST Buffer Kit is also available containing all buffers needed for purification of GST-tagged.

4. Principle and characteristics

GST GraviTrap contains 2 ml Glutathione Sepharose 4B medium/column, which has high binding capacity for GST-tagged proteins. Table 1 summarizes GST GraviTrap characteristics.

Table 1. GST GraviTrap characteristics.

Column material	Polypropylene barrel, polyethylene frits
Column volume	13 ml
Medium	Glutathione Sepharose 4B
Average bead size	45–165 µm
Ligand	Glutathione and 10-carbon linker arm
Ligand concentration	7–15 µmol glutathione/ml medium
Protein binding capacity[1]	Approx. 10 mg recombinant glutathione S-transferase (M_r 26 000)/ml medium (protein dependent)
Bed volume	2 ml
Compatibility during use	All commonly used aqueous buffers
Chemical stability	No significant loss of the capacity is detected when Glutathione Sepharose 4B is exposed to 0.1 M citrate (pH 4.0), 0.1 M NaOH, 70% ethanol or 6 M guanidine hydrochloride[2] for 2 hours at room temperature. No significant loss of
Chemical stability (continued)	binding capacity is observed after exposure to 1% SDS for 14 days.

Storage solution	20% Ethanol
pH stability	4 to 13
Storage temperature	4°C to 30°C

Note: It is not recommended to autoclave the gel.

[1] Binding capacity is protein-dependent. The binding of GST-tagged proteins depends on size, conformation, and concentration of the protein in the sample. Binding of GST to glutathione is also flow dependent, and lower flow rates often increase the binding capacity. This is important during sample loading. Protein characteristics, pH, and temperature may also affect the binding capacity.

[2] Exposing the sample to 6 M guanidine hydrochloride will denaturate the GST-tag. It is therefore important to remove all guanidine hydrochloride before use.

5. Buffers

Binding buffer
10 mM PBS, pH 7.4 (10 mM Na_2HPO_4, 140 mM NaCl, 2.7 mM KCl, 1.8 mM KH_2PO_4, pH 7.4)

Elution Buffer
50 mM Tris-HCl, 10 to 20 mM reduced glutathione, pH 8.0

Note: 1 to 20 mM DTT may be included in the binding and elution buffers to increase the purity. However, this may result in lower yield of GST-tagged protein

6. Sample pretreatment

Cell lysis

For larger-scale cultures, mechanical lysis with either sonication or homogenizers is the most common and also a recommended methods to lyse cells. However, the process is only efficient for cell suspensions greater than 2 ml. While screening of multiple samples can be performed on aliquots of such larger volume, the growth and processing of a large number of cultures of this size may be inconvenient. Chemical lysis with commercial lysis kits is a third alternative and simplify the process when lysis of multiple samples needs to be purified. There are several commercial lysis kits on the market with variable efficiency and lysis should be performed according to protocols for each method. GE Healthcare can provide lysis kits to different expression systems, such as Mammalian Protein Extraction Buffer kit for mammalian expression systems and Yeast Protein Extraction Buffer for yeast expression systems. For bacteria, there are several chemical lysis kits available on the market. For small-scale cultures, chemical lysis with commercial lysis kits and freeze/thaw cycles are recommended to be used as lysis methods.

Note: Cell culture lysates may be directly applied to the column without prior clarification.

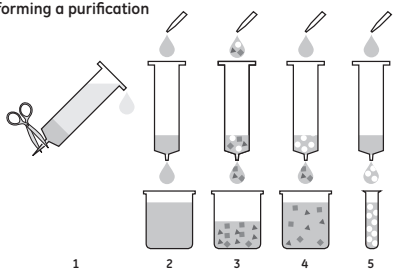
7. Purification protocol

7.1. LabMate

Connecting LabMate™ reservoir (Code No. 18-3216-03) to the column increases convenience when handling volumes above 10 ml. This raises the loading capacity to approx. 35 ml.

7.2. Protocol

Performing a purification



1. Cut of the bottom tip. Remove the top cap and pour of the excess liquid.

2. Wash the column with 10–20 ml of 10 mM PBS pH 7.4 to remove the preservative.

Note: For increased convenience, use GST Buffer Kit (28-9523-61) and follow the protocol for preparing the different buffers, or prepare buffers manually.

3. Apply the sample to the column. If needed, clarify the sample by centrifugation. The sample may be diluted in PBS if it is too concentrated. If large volumes is needed use LabMate reservoir.

4. Wash the column with 2 × 10 ml of PBS.

5. Elute the bound material with 10 ml of elution buffer (10 mM Glutathione in 50 mM Tris-HCl pH 8.0) and collect 1–2 ml fractions.

7.3. Regeneration and storage

The gel may be regenerated by washing the column with high salt buffer (PBS + 3 M NaCl. For longer storage, wash the column with 2 × 5 (2 × 10 ml) bed volumes of PBS and the 2 × 5 (2 × 10 ml) bed volumes of 20% ethanol Store at 4°C to 30°C.

8. Appendixes

8.1. Preparation of buffers

The GST Buffer Kit (28-9523-61) can be used for increased convenience. However, buffers could also be prepared according to the following protocol.

Water and chemicals used for buffer preparation should be of high purity.

Binding buffer:
Prepare 250 ml binding buffer with a final concentration of 10 mM sodium phosphate, 140 mM NaCl, pH 7.4.

1. Take 0.22 g $Na_2HPO_4 \times 2H_2O$ (177.99 g/mol), 0. 17 g $NaH_2PO_4 \times H_20$ (127.99 g/mol) and 2.04 g NaCl (58.44 g/mol).

2. Add distilled water to 200 ml and dissolve completely.

3. Adjust to pH 7.4 with HCl.

4. Add distilled water to 250 ml.

Elution buffer:
Prepare 100 ml of 50 mM Tris-HCl, 10 mM glutathione, pH 8.0:

1. Add the following into a calibrated bottle:
- 606 mg Tris(hydroxymethyl)-aminomethan (121.14 g/mol).
- 308 mg Reduced glutathione (307.30 g/mol).

2. Add distilled water to 80 ml and dissolve completely.

3. Adjust pH from basic to 8.0 with HCl.

4. Add distilled water to 100 ml.

5. If needed, add a reducing agent (1–20 mM, depending on sample).

Note: Reducing agents, e. g. DTT, DTE, TCEP and β-mercaptoethanol, needs to be fresh. Add, therefore, the reducing agent to the sample and buffers just prior to equilibration of the wells.

8.2. Detection of GST GST-tagged proteins

Products provided of GE Healthcare

The GST detection modules (exists as both single and 96 well detection modules) provides convenient format for rapidly determination/screening of GST-tagged proteins. The module uses immobilized goat polyclonal Anti-GST Antibody to capture GST-tagged proteins from complex mixtures and exhibit very low, non-specific background binding. Using a chromogenic substrate, the system can detect as little as 1 ng of recombinant GST, providing a level of sensitivity that is 10 to 100 times greater than capture plates using immobilized glutathione. Anti-GST Antibody is also available as a stand alone product in 0.5 ml suitable for 50 detections.

CDNB assay

In addition to SDS-PAGE analysis of recombinants, the relative level of expression of GST-tagged protein can be estimated using the GST substrate CDNB (1-chloro-2,4-dinitrobenzene) which is included in the GST Detection Module.

Additional analyses with GST detection module

If recombinants expressing tagged proteins cannot be identified using the methods described above, clones can also be identified by ECL™ GST Western Blotting Detection Kit for analysis using the Anti-GST Antibody contained in the GST detection module or available as a stand-alone product. Another alternative is to perform a functional assay, if available, specific for the protein of interest. The yield of tagged protein can be estimated by measuring the absorbance at 280 nm. The amount of GST affinity tag can be approximated by 1 $A_{280} \approx 0.5$ mg/ml (This value is based on the extinction coefficient of the GST monomer using a Bradford protein assay. Other protein determination methods may produce different extinction coefficients). The yield of protein may also be determined by standard chromogenic methods (e.g. Lowry, BCA, Bradford, etc.). If a Lowry

or BCA type method is to be used, the sample must first be dialyzed against 2 000 volumes of 10 mM PBS to remove glutathione, which can interfere with protein measurement. The Bradford method can be performed in the presence of glutathione.

SDS-PAGE electrophoresis with Coomassie staining. If increased sensitivity is needed use Deep Purple staining

1. Transfer 10 µl aliquots of each sample to be analyzed (e.g. samples retained following cell resuspension and lysis, column flow through, washes, eluates, etc.) to fresh tubes.
2. To each sample, add 30 µl SDS loading buffer (**Note:** The volume depends on the concentration of SDS in loading buffer). Vortex briefly and heat for 5 minutes at 90°C to 100°C.
3. Load the samples onto a 10–12.5% SDS-polyacrylamide gel.
4. Run the gel for the appropriate time and stain with Coomassie blue/deep purple according to existing protocols to visualize the parental GST (synthesized in control cells carrying the parental pGEX vector) and the tagged protein.

Note: Transformants expressing the desired tagged protein will be identified by the presence of a novel tagged protein larger than the M_r 29 000 size of parental GST. If the above analysis indicates that the tagged protein has adsorbed to the Glutathione Sepharose 4B, you may proceed to large-scale purification. If the tagged protein is absent from the purified material, it may be insoluble or expressed at very low levels; refer to the troubleshooting guide (page 15) for a discussion of this problem. Interpretation is sometimes complicated when tagged proteins break down and release the M_r 26 000 GST moiety. Such cases are usually recognized by the reduced level of the full size GST-tagged protein, and by the series of larger, partial proteolytic fragments down to M_r 26 000.

* Parental pGEX vectors produce a M_r 29 000 GST-tagged protein containing amino acids coded for the pGEX multiple cloning site.

9. Troubleshooting

Consult the GST Gene Fusion System Handbook for more detailed information of the suggestion of solutions given below; see ordering information and pGEX instructions regarding troubleshooting recommendations for expression, fermentation, and solubilization.

Problem: No GST-tagged protein is detected by Coomassie-stained SDS gel of the bacterial sonicate.

Possible causes/solutions

1. Optimize expression conditions.
Optimization of expression conditions can dramatically improve yields. Investigate the effects of cell strain, medium composition, incubation temperature and induction conditions on GST-tagged protein yield. Exact conditions will vary for each GST-tagged protein.

2. Check DNA sequences.
It is essential that protein-coding DNA sequences be cloned in the proper translation frame in pGEX vectors. Cloning junctions should be sequenced using 5′ pGEX sequencing primer and 3′ pGEX sequencing primer to verify that inserts are in-frame with GST. The reading frame of the multiple cloning site for each pGEX vector is shown GST Gene Fusion System Handbook.

3. Analyze a small aliquot of an overnight culture by SDS-PAGE. Generally, a highly expressed protein will be visible by Coomassie staining when 5–10 µl of an induced culture whose A_{600} is ~ 1.0 is loaded on the gel. Non-transformed host *E. coli* cells and cells transformed with the parental pGEX vector should be run in parallel as negative and positive controls, respectively. The presence of the GST-tagged protein in this total cell preparation and its absence from a clarified sonicate may indicate the presence of inclusion bodies (see page 16–17).

4. Check for expression by immunoblotting.
Some GST-tagged proteins may be masked on an SDS-polyacrylamide gel by a bacterial protein of approximately the same molecular weight. Immunoblotting can be used to identify tagged proteins in these cases. Run an SDS-poly acrylamide gel of induced cells as above and transfer the proteins to a nitrocellulose or PVDF membrane. Detect GST-tagged protein using Anti-GST Antibody (included in the GST Detection Module).

Problem: Majority of fusion GST-tagged is found in post-lysis pellet.

Possible causes/solutions

SDS-PAGE analysis of samples collected during the preparation of the bacterial lysis may indicate that the majority of the GST-tagged protein is located in the pellet. Possible causes and solutions are discussed below.

1. Lysis may be insufficient.
Cell disruption is evidenced by partial clearing of the suspension or may be checked by microscopic examination. If the lysate is too viscous for handling, DNase I may be added to a final concentration of 10 µg/ml during lysozyme treatment.

2. GST-tagged protein may be insoluble (inclusion bodies).
If insufficient protein is found in the soluble fraction following centrifugation of the sonicate, it may be necessary to alter growth conditions:

- GST-tagged protein solubility can be dramatically increased by lowering the growth temperature during induction. Experiment with growth temperatures in the range of 20°C to 30°C.
- Alter level of induction by decreasing IPTG concentration to < 0.1 mM.

- Alter timing of induction.
- Induce for a shorter period of time.
- Induce at a higher cell density for a short period of time.
- Increase aeration. High oxygen transport can help prevent the formation of inclusion bodies.

It may be necessary to combine the above approaches. Exact conditions must be determined empirically for each tagged protein. If the above techniques do not significantly improve expression of soluble tagged protein, protein can be solubilized from inclusion bodies using common denaturants such as 4–8 M guanidine hydrochloride, 4–8 M urea, detergents, alkaline pH (> 9), organic solvents, N-lauroyl-sarcosine. Other variables that affect solubilization include time, temperature, ionic strength, ratio of denaturant to protein and the presence of thiol reagents.

Following solubilization, proteins must be properly refolded to regain function. Denaturant can be removed by dialysis, dilution, or gel filtration to allow refolding of the protein and formation of the correct intramolecular associations. Critical parameters during refolding include pH, presence of thiol reagents and the speed of denaturant removal. Once refolded, protein may be purified by ion exchange, gel filtration or affinity chromatography. GST-tagged proteins can be purified to some extent while denatured. In some instances where GST-tagged proteins formed inclusion bodies, solubilization and binding to Glutathione Sepharose 4B was achieved in the presence of 2–3 M guanidine hydrochloride or urea. Success has also been achieved using up to 2% Tween 20 for solubilization and binding. Binding to Glutathione Sepharose 4B can also be achieved in the presence of 1% CTAB, 10 mM DTT or 0.03% SDS. Success of affinity purification in the presence of these agents will depend on the nature of the fusion protein.

Problem: GST-tagged protein does not bind to Glutathione Sepharose 4B.

Possible causes/solutions

1. Poor equilibration of GST GraviTrap before use.
Check that the GST GraviTrap column has been equilibrated with a buffer between pH 6.5 to 8.0 (e.g. PBS) before the cell lysate is applied. Binding of GST-tagged proteins to Glutathione Sepharose 4B is not efficient at pH less than 6.5 or greater than 8.

2. Too short incubation time.
GST-tagged proteins have a slow kinetic towards Glutathione Sepharose 4B and it may be necessary to increase the incubation time for the sample.

3. Low expression level of the protein.
The binding capacity is concentration dependent. GST-tagged proteins expressed at low levels have generally a poor binding affinity to Glutathione Sepharose 4B. Concentrate the sample before adding it to GST GraviTrap.

4. Test binding of GST from parental pGEX.
Prepare a lysate of cells containing the parental pGEX plasmid and check binding to the matrix. If GST produced from the parental plasmid binds with high affinity, then the fusion partner 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 the number of washes.

5. Masked binding site.
The addition of DTT to a final concentration of 5 mM prior to cell lysis can significantly increase binding of some GST-tagged proteins to Glutathione Sepharose 4B.

6. Too extensive sonication/mechanical lysis
Too extensive sonication/mechanical lysis may denaturize the GST-tag which then prevents binding.

Use as mild conditions as possible during cell lysis.

Problem: GST-tagged protein is not eluted from Glutathione Sepharose 4B.

Possible causes/solutions

1. Insufficient elution. Increase the duration of elution. In some instances, overnight elution at room temperature or 4°C is most effective.

2. Increase the volume of elution buffer.
Note that Glutathione Sepharose 4B will also function as a gel filtration medium with an approximate molecular weight exclusion limit of M_r 1 to 107. Small proteins (especially those liberated following cleavage with a site-specific protease) may require large elution volumes. Proteins eluted in a large volume may require concentration by ultra filtration.

3. Increase the concentration of glutathione in the elution buffer.
General protocols use 10 mM glutathione for elution which should be sufficient for most applications. Additional reduced glutathione must be obtained separately. Keep in mind that if the glutathione concentration is significantly increased above 15 mM, the buffer concentration will have to be increased to maintain proper pH.

4. Increase the ionic strength of the elution buffer.
The addition of 0.1–0.2 M NaCl to the elution buffer may also improve results. However, proteins that are very hydrophobic may precipitate in the presence of high salt concentrations; here, addition of a non-ionic detergent may improve results (see page 20).

5. Add a non-ionic detergent to the elution buffer.
Nonspecific hydrophobic interactions may prevent solubilization and elution of fusion proteins from Glutathione Sepharose 4B. Addition of a non-ionic detergent can improve results. The addition of 0.1% Triton X-100 or 2% N-octyl gluco side can significantly improve elution of some GST-tagged proteins.

Problem: Insufficient purity of GST-tagged protein

Possible causes/solutions

1. Purification of a GST-tagged protein from *E. coli* sample which results in multiple bands after electrophoresis/ Western blotting analysis of eluted target protein.
A M_r 70 000 protein is co-purifying with the GST-tagged protein
The M_r 70 000 protein may be 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 GST SpinTrap™. Alternatively, remove the DnaK protein by passing the tagged protein solution through ATP-agarose or by ion exchange.

2. Partial degradation of tagged protein by proteases in lysate
Add a protease inhibitor. Adding 1 mM PMSF or Pefabloc™ SC to the lysis solution may improve results.

Note: Serine protease inhibitors must be removed prior to cleavage by thrombin or factor Xa. PreScission™ Protease is not a consensus serine protease and is insensitive to many of the protease inhibitors tested at GE Healthcare.

3. Host strain is not protease deficient
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 protease-deficient strain may be required (e.g. *lon*- or *ompT*). *E. coli* BL21 is provided with the pGEX vectors. This strain is *ompT*.

10. Ordering information

Product	Quantity	Code No
GST GraviTrap	10 × columns	28-9523-60
Accessories	**Quantity**	**Code No**
LabMate PD-10 Buffer Reservoir	10	18-3216-03
GST Buffer Kit	1	28-9523-61
PD-10 Desalting Columns	30	17-0851-01
Related product	**Quantity**	**Code No**
GST Bulk Kit	1	27-4570-01
GSTrap™ 4B	5 × 1 ml	28-4017-45
GSTrap 4B	100 × 1 ml[1]	28-4017-46
GSTrap 4B	1 × 5 ml	28-4017-47
GSTrap 4B	5 × 5 ml	28-4017-48
GSTrap 4B	100 × 5 ml[1]	28-4017-49

[1] Pack size available by specific customer order

Small scale product	Quantity	Code No
GST SpinTrap	50 columns	28-9523-59
GST MultiTrap™ 4B	4 × 96-well plates	28-4055-00
GST MultiTrap 4 FF	4 × 96-well plates	28-4055-01
GST detection product	**Quantity**	**Code No**
GST Detection Module	50 detections	27-4590-01
GST Detection Module (96-well format)	5 × 96-well plates	27-4592-01
Anti-GST Antibody	0.5 ml, 50 detections	27-4577-01
ECL GST Western Blotting Detection Kit	1	RPN123

GST cloning product	Quantity	Code No
pGEX 5' Sequencing Primer 5'-d [GGG-CTG-GCAAGCCACGTTTGGTG]-3´	0.05 A_{260} units	27-1410-01
pGEX 3' Sequencing Primer 5'-d [CCG-GGAGCT-GCATGTGTCAGAGG]-3'	0.05 A_{260} units	27-1411-01
E. coli BL21	1 vial	27-1542-01
13 different pGEX vectors	5 µg or 25 µg	*

* See www.gelifesciences.com

Site-specific Proteases	Quantity	Code No
PreScission Protease	500 units	27-0843-01
Thrombin	500 units	27-0846-01
Factor Xa	400 units	27-0849-01

Lysis kit	Quantity	Code No
Yest Protein Extraction Buffer Kit	1	28-9440-45
Mammalian Protein Extraction Buffer	1	28-9412-79

Literature	Code No
GST Gene Fusion System Handbook	18-1157-58
Recombinant Protein Purification Handbook, Principles and methods	18-1142-75
Affinity Chromatography Handbook, Principles and methods	18-1022-29

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