cytiva

GST **SpinTrap**

50 prepacked GST SpinTrap columns

Product Booklet

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1 Introduction

Product code

28952359

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

All chemicals should be considered as potentially hazardous. For use and handling of the products in a safe way, refer to the Safety Data Sheets.

Storage

Store at 4°C to 30°C. Do not freeze.

2 Description

GST SpinTrap™ columns are designed for rapid small-scale purification of Glutathion-S-Transferase (GST)-tagged proteins. The GST SpinTrap features rapid, mild affinity purification of GST-tagged proteins and > 90% purity can be obtained in a single step. GST-tagged proteins are recovered from the matrix under mild elution conditions (10 mM glutathione) which preserve the functionality of the proteins.

The columns are suitable for purification of multiple samples in parallel, for example in screening experiments such as evaluation of the media, growth temperature, culture density, induction conditions and other variables to find the best conditions for expression.

The GST SpinTrap provides 50 prepacked Glutathione Sepharose™ 4B columns in a convenient microspin format. Each microspin column contains 50 µL bed volume of Glutathione Sepharose 4B, enough to purify up to 500 µg of recombinant GST (rGST). The capacity will vary with the nature of the GST-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 proteins.

3 Principle and characteristics

GST SpinTrap contains 50 µL Glutathione Sepharose 4B medium/column, which has high binding capacity for GST-tagged proteins.

Table 1, on page 5 summarizes GST SpinTrap characteristics.

Table 1. GST SpinTrap characteristics

Column material	Polypropylene barrel, polyethylene frits
Column volume	900 µL
Medium	Glutathione Sepharose 4B
Average bead size	90 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	50 µL
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 binding capacity is observed after exposure to 1% SDS for 14 days.
Storage solution	PBS and 0,05% Kathon® CG/ICP Biocide
pH stability	4 to 13
Storage temperature	Room temperature

[1] Binding capacity is protein-dependent. The binding of GST-tagged proteins depends on size, conformation, and concentration of the protein in the sample loaded. 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 with 6 M guanidine hydrochloride will denaturate the GST-tag. It is therefore important to remove all guanidine hydrochloride before use.

4 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.*

5 Sample pretreatment

Cell lysis

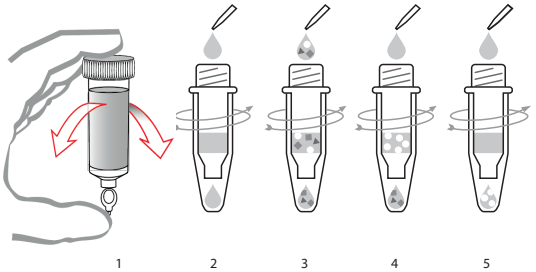
For small-scale cultures, freeze/thaw or chemical lysis with commercial kits are recommended for cell lysis. There are several commercial lysis kits on the market with variable efficiency and lysis should be performed according to protocols for each method. Cytiva can provide lysis kits to different expression systems, Mammalian Protein Extraction Buffer for mammalian expression systems and Yeast Protein Extraction Buffer Kit for yeast expression systems. For bacteria there are several chemical lysis kits available on the market. For larger-scale cultures, mechanical lysis with either sonication or homogenizers is recommended to lyse cells. However, the process is only efficient for cell suspensions greater than 2 mL, representing culture volumes of at least 40 mL.

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

6 Purification protocols

The capacity of each SpinTrap column is 500 µg GST-tagged protein and maximum 600 µL of culture lysate or buffer can be applied to a SpinTrap column. This represents a volume of lysate produced from a 12 mL culture. The following procedure is designed to accommodate lysates prepared from 2–12 mL of culture. If multiple samples containing GST-tagged proteins need to be purified, e.g. expression screening, GST MultiTrap™ 4B or GST MultiTrap FF, prepacked 96-well filter plates, can be used to increase the throughput.

Performing a purification



Run purifications on GST SpinTrap using a standard microcentrifuge. Place the column in a 2 mL microcentrifuge tube to collect the liquid during centrifugation. Use a new 2 mL tube for every step (steps 1–5).

Step	Action
1	Resuspend the resin in each GST SpinTrap column by inverting and shaking the column repeatedly. Loosen the top cap one-quarter of a turn and twist off the bottom closure. Place the column in a 2 mL microcentrifuge tube and centrifuge for 30 seconds at 100 × g (approx. 1500 rpm in an Eppendorf™ 5415R, 24 position fixed-angle rotor) to remove the storage liquid. ***Note:*** *It is important that each column is appropriately labeled with the sample being applied to it, as their order may be mixed in subsequent steps.*
2	Remove and discard the top cap. Equilibrate the column by adding 600 µL binding buffer. Centrifuge for 30 seconds at 100 × g.
3	Add the sample (see Chapter Sample pretreatment). Maximum sample volume is 600 µL in one go. Mix gently at room temperature for 5–10 minutes to ensure optimal binding of GST-tagged proteins to the Glutathione Sepharose 4B medium. Centrifuge for 30 seconds at 100 × g. ***Note:*** *Several sample applications can be performed as long as the capacity of the column is not exceeded.*
4	Wash with 600 µL binding buffer. Centrifuge for 30 seconds at 100 × g. Repeat the wash step once.

Step	Action
5	Elute the target protein twice with 200 µL elution buffer. Centrifuge for 30 seconds at 100 × g and collect the purified sample. The first 200 µL will contain the majority of the target protein. ***Note:*** *Yields of GST-tagged protein may be increased by repeating the elution step two or three times and pooling all eluates.*

7 Appendices

Preparation of buffers

GST Buffer Kit (28952361) 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:

Step	Action
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.

Step	Action
3	Adjust to pH 7.4 with 1 M HCl.
4	Add distilled water to 250 mL.

Elution buffer:

Prepare 100 mL of 50 mM Tris-HCl, 10 mM glutathione, pH 8.0:

Step	Action
1	Add the following into a calibrated bottle: **a.** 606 mg Tris(hydroxymethyl)-aminomethane (121.14 g/mol). **b.** 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 1 M 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.*

Scale up of purification

After purification screening on GST SpinTrap it may be necessary to scale up the purification. Cytiva can provide several different products which can be used both manually, i.e gravity flow and with syringe/peristaltic pump and with chromatography systems such as ÄKTA™ design systems, i.e. ÄKTAprime, ÄKTAexplorer and ÄKTAxpress. GST GraviTrap™ is a gravity column prepacked with 2 mL Glutathione Sepharose 4B and GSTrap™ 4B 1 mL and GSTrap 4B 5 mL columns also prepacked with Glutathione Sepharose 4B are used with either a syringe/peristaltic pump or in chromatography systems. More information about the products can be found in the instructions of the products, respectively. The *GST Gene Fusion System Handbook* or *cytiva.com* can give valuable tips using the GST Fusion system and more detailed information about products.

Detection of GST-tagged proteins

Products provided by Cytiva

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, nonspecific 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 GST-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 ofGST-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 use Deep Purple staining

Step	Action
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 (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 GST-tagged protein. ***Note:*** *Transformants expressing the desired GST-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 fusion 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 Chapter for a discussion of this problem. Interpretation is*

Step	Action
	sometimes complicated when fusion 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 tagged protein, and by the series of larger, partial proteolytic fragments down to M_r 26 000.
	Note: *Parental pGEX vectors produce a M_r 29 000 GST-tagged protein containing amino acids coded for the pGEX multiple cloning site.*

8 Troubleshooting

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

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

Possible causes/solutions

- 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 fusion protein yield. Exact conditions will vary for each fusion protein.

- 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 in the *GST Gene Fusion System Handbook*.

- 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 fusion protein in this total cell preparation and its absence from a clarified sonicate may indicate the presence of inclusion bodies. (See *Problem: Majority of GST-tagged protein is found in post-lysis pellet, on page 16*)

- Check for expression by immunoblotting.

  Some fusion 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 fusion proteins in these cases. Run an SDS-polyacrylamide 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 GST-tagged protein 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 theGST-tagged protein is located in the pellet. Possible causes and solutions are discussed below.

- 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.

- Fusion 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:

  - Fusion 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 fusion 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-lauroylsarcosine. 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. Fusion 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 tagged protein.

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

Possible causes/solutions

- Poor equilibration of GST SpinTrap before use.

  Check that the GST SpinTrap 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.

- 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.

- Low expression level of the protein.

  The binding capacity is concentration dependent. GST-tagged proteins expressed at low levels generally have a poor binding affinity to Glutathione Sepharose 4B. Concentrate the sample before adding it to the GST SpinTrap.

- 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.

- 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.

- 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: Fusion protein is not eluted from Glutathione Sepharose 4B

Possible causes/solutions

- Insufficient elution.

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

- 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 2 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.

- 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.

- Increase the ionic strength of the elution buffer.

  The addition of 0.1 to 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 below).

- Add a non-ionic detergent to the elution buffer.

  Nonspecific hydrophobic interactions may prevent solubilization and elution of GST-tagged proteins from Glutathione Sepharose 4B. Addition of a non-ionic detergent can improve results. The addition of 2% N-octyl glucoside can significantly improve elution of some GST fusion proteins.

Problem: Insufficient purity of GST-tagged protein

Possible causes/solutions

- Purification of a GST-tagged protein from an *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.

- 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 Cytiva.*

- 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*.

9 Ordering Information

Product	Quantity	Product code
GST SpinTrap	50 columns	28952359

Related product	Quantity	Product code
GSTBuffer Kit	1	28952361
GST MultiTrap 4B	4 × 96 well plates	28405500
GST MultiTrap FF	4 × 96 well plates	28405501
PD-10 Desalting columns	30	17085101

GST detection product	Quantity	Product code
GST Detection Module	50 detections	27459001
GST Detection Module (96-well format)	5 × 96-well plates	27459201
Anti-GST Antibody	0.5 mL, 50 detections	27457701
ECL GST Western Blotting Detection Kit	1	RPN123

GST cloning product	Quantity	Product code
[GGGCTGGCAAGCCACGTTTGGTG]-3′ pGEX 3′	0.05 A_{260} units	27141001
Sequencing Primer 5′-d[CCGGGAGCTGCATGTGTCAGAGG]-3′	0.05 A_{260} units	27141101
E. coli BL21	1 vial	27154201
13 different pGEX vectors	5 µg or 25 µg	See *cytiva.com*

Site-specific Proteases	Quantity	Product code
PreScission Protease	500 units	27084301
Thrombin	500 units	27084601

Site-specific Proteases	Quantity	Product code
Factor Xa	400 units	27084901

Lysis kit	Quantity	Product code
Yeast Protein Extraction Buffer Kit	1	28944045
Mammalian Protein Extraction Buffer	1	28941279

Scale up column	Quantity	Product code
GST Bulk Kit	1	27457001
GST GraviTrap	10 × columns	28952360
GSTrap 4B	5 × 1 mL	28401745
GSTrap 4B	100 × 1 mL[1]	28401746
GSTrap 4B	1 × 5 mL	28401747
GSTrap 4B	5 × 5 mL	28401748
GSTrap 4B	100 × 5 mL[1]	28401749

[1] Pack size available by specific customer order.

Literature	Product code
GST Gene Fusion System Handbook	18115758
Recombinant Protein Purification Handbook, Principles and methods	18114275
Affinity Chromatography Handbook, Principles and methods	18102229

cytiva.com/sampleprep

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