

pGEX-4T-1 GST Expression Vector

Product Specification Sheet

Introduction

Product code

28954549

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

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

Handling

The vector must be removed from the dry-ice packaging and stored at -20°C. After thawing, centrifuge briefly to recover contents.

Expiry

Vector is stable for a minimum of 8 weeks from date of receipt when stored under recommended storage conditions.

Components

25 µg vector supplied in 10 mM Tris, 1 mM EDTA pH 8.0.

Quality control

Purified plasmid will contain predominantly supercoiled form at typically greater than 90% by agarose gel electrophoresis. Chromosomal DNA from the host is not observed. Plasmid is assayed to demonstrate presence of Bam H1; EcoRI; Not I restriction endonuclease sites.

Protocols

Prepare fusion construct by inserting gene of interest into the multiple cloning site of pGEX-4T-1 using any one, or combination of unique restriction sites and transform into a host of choice such as *E. coli* BL21 (27154201).

Growth and Induction

Step	Action
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1	Dilute an overnight culture transformed with pGEX fusion construct, 1:10 in fresh complex medium containing 100 µg/mL ampicillin. Grow the cells at 37°C to mid-log phase ($A_{600} = 0.6-1.0$).
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Step	Action
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2	Induce expression of fusion proteins by adding isopropyl-β D-thiogalactoside (IPTG) to 0.1 mM final concentration and allow the cells to grow for an additional 3–5 hours at 37°C.
3	Expression of GST fusion proteins can be monitored using the Anti-GST Antibody (27457701), GST Detection Modules (27459001, 27459201) or ECL™ GST Western Blotting Detection Kit (RPN1237).

Preparation of cell extracts

Step	Action
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1	Sediment the cells by centrifugation and resuspend in 1/20 volume of PBS (PBS: 140 mM NaCl, 2.7 mM KCl, 10 mM Na ₂ HPO ₄ , 1.8 mM KH ₂ PO ₄ , pH 7.3).
2	Lyse the cells by mild sonication or chemical lysis.
3	Add detergent to a final concentration of 1% and mix gently at room temperature (25°C) for 30 minutes to solubilize proteins.
4	Centrifuge the crude extract at 10 000 × g for 5 minutes at 4°C.

Purification

There are a range of Glutathione Sepharose™ prepacked column and bulk media products available to purify GST Fusion proteins. For manual purification of sample volumes up to 600 µL use GST SpinTrap™ microspin columns or GST MultiTrap™ 4B 96-well plates. For sample volumes between 600 µL and 10 mL use GST GraviTrap™ gravity flow column. Where sample volumes are above 10 mL, use LabMate reservoir together with GST GraviTrap. All formats described can be used for preparation of samples in parallel. In addition GST HiTrap™ 1 and 5 mL columns and GST HiPrep™ FF 16/10 column are available for purification in a chromatography system such as the ÄKTA™ design system. Alternatively, Glutathione Sepharose bulk media are available from 10 mL up to 500 mL. A GST Bulk Kit is also available combining 10 mL Glutathione Sepharose 4B bulk medium and 5 empty columns with required buffers. For simplified buffer preparation use the GST Buffer Kit. Ordering information for all associated products is listed below.

Site-specific proteolysis of fusion proteins

Separation of the recombinant protein from the glutathione S-transferase moiety may be accomplished by site specific proteolysis using bovine thrombin (27084601). Exact reaction conditions will vary with the nature of the fusion protein. The following conditions may be used as a guideline and should be optimized for each fusion protein: thrombin concentration, 0.2% (w/w) of fusion protein; reaction buffer, PBS; incubation temperature, 25°C; incubation time, 2–16 hours (1, 2).

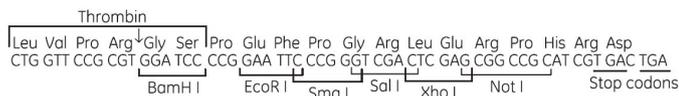


Fig 1. Multiple Cloning region and protease cleavage site.

For more information on the use of pGEX vectors, see *GST Gene Fusion System Handbook*.

Intracellular expression of some eukaryotic proteins in *Escherichia coli* can lead to the formation of inclusion bodies (2). Increased solubilities can be obtained by lowering the growth temperature from 37°C to 28–30°C (3). Shortening the induction period may also improve results. Exact conditions must be established for each protein.

The following primers for double-stranded sequencing of pGEX vectors are available: 5' pGEX Sequencing Primer (bases 869–891) and 3' pGEX Sequencing Primer (bases 1020–998).

Further information relating to DNA sequence, restriction maps and control regions can be found at: cytiva.com

References

1. Smith, D. B. and Johnson, K. S., *Gene* 67, 31 (1988).
2. Eaton, D., et al., *Biochemistry* 25, 505 (1986).
3. Schein, C. H. and Noteborn, M. H. M., *Bio/Technology* 6, 291 (1988).
4. Smith, D. B. and Corcoran, L. M., *Current Protocols*, pg. 16.7.1 (1990).

Related products

GST vector products	Product code
pGEX-4T-2 (25 µg)	28954550
pGEX-4T-3 (25 µg)	28954552
pGEX-5X-1 (25 µg)	28954553
pGEX-5X-2 (25 µg)	28954554
pGEX-5X-3 (25 µg)	28954555
pGEX-2TK (25 µg)	28954646
pGEX-6P-1 (25 µg)	28954648
pGEX-6P-2 (25 µg)	28954650
pGEX-6P-3 (25 µg)	28954651
pGEX-2T (25 µg)	28954653
pGEX-3X (25 µg)	28954654
pGEX-1IT EcoR/BAP (5 µg)	28954656
pGEX 5' Sequencing Primer 5'-d[GGG-CTGGCAAGCCACGTTTGGTG]-3'	27141001
pGEX 3' Sequencing Primer 5'-d[CCG-GGAGTGCATGTGTGTCAGAGG]-3'	27141101

GST vector products	Product code
<i>E. coli</i> BL21 1 vial	27154201

GST purification products	Product code
GST GraviTrap (10 columns)	28952360
LabMate PD-10 Buffer Reservoir (50)	18321603
GST Buffer Kit	28952361
GST Bulk Kit	27457001
GST SpinTrap (50 columns)	28952359
GST MultiTrap 4B (4 × 96-well plates)	28405500
GST MultiTrap 4 FF (4 × 96-well plates)	28405501
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
Glutathione Sepharose 4B (10 mL)	17075601
Glutathione Sepharose 4B (100 mL)	17075605
Glutathione Sepharose 4B (300 mL)	17075604

¹ Pack size available by specific customer order.

GST detection product	Product code
GST Detection Module	27459001
GST Detection Module (96-well format)	27459201
Anti-GST Antibody	27457701
ECL GST Western Blotting Detection Kit	RPN1237

Site-specific Proteases	Product code
PreScission Protease (500 units)	27084301
Thrombin (500 units)	27084601
Factor Xa (400 units)	27084901

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

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

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