GE Healthcare

pGEX-3X
GST Expression Vector

Product Specification Sheet

Code: 28-9546-54

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.

Handling
The vector should be removed from the dri-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.

Safety warnings and precautions
All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

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. Chromasomal DNA from the host is not observed. Plasmid is assayed to demonstrate presence of B*am* H1; *Eco*R I; *Sma* I restriction endonuclease sites.

Protocols
Prepare fusion construct by inserting gene of interest into the multiple cloning site of pGEX-3X using any one, or combination of unique restriction sites and transform into a host of choice such as *E. coli* BL21 (27-1542-01).

Growth and Induction:

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).
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 (27-4577-01), GST Detection Modules (27-4590-01, 27-4592-01) or ECL GST Western Blotting Detection Kit (RPN1237).

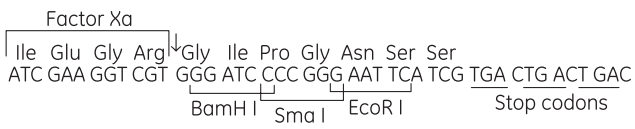
Preparation of cell extracts:

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_2HPO_4, 1.8 mM KH_2PO_4, pH 7.3).
2. Lyse the cells by mild sonication or chemical lysis.
3. Add Triton X-100 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 Gluthatione 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™ 16/10 column are available for purification in a chromatography system such as the ÄKTA™ design system. Alternatively, Gluthatione Sepharose bulk media are available from 10 ml up to 500 ml. A GST Bulk Kit is also available combining 10 ml Gluthatione Sepharose 4B bulk medium 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:
Site-specific proteolysis of fusion proteins expressed from pGEX-3X may be acomplished using factor Xa (1) via recognition sequence adjacent to the multiple cloning region. Exact reaction conditions for factor Xa cleavage 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: factor Xa concentration, 1% (w/w) of fusion protein; reaction buffer [50 mM Tris (pH 7.5), 150 mM NaCl, 1 mM $CaCl_2$]; incubation temperature, 25°C; incubation time, 1 hour. The molecular weight of factor Xa (bovine) is approximately 48 kDa.

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:
http://www.gelifesciences.com

References

1. Nagai, and Thogersen, *Nature* **309**, 810 (1984).
2. Schein, C. H. and Noteborn, M. H. M., Bio/Technology **6**, 291 (1988).
3. Smith, D. B. and Corcoran, L. M., Current Protocols, pg. 16.7.1 (1990).

Related products

GST vector products	**Code No.**
pGEX-4T-1 (25 µg)	28-9545-49
pGEX-4T-2 (25 µg)	28-9545-50
pGEX-4T-3 (25 µg)	28-9545-52
pGEX-5X-1 (25 µg)	28-9545-53
pGEX-5X-2 (25 µg)	28-9545-54
pGEX-5X-3 (25 µg)	28-9545-55
pGEX-2TK (25 µg)	28-9546-46
pGEX-6P-1 (25 µg)	28-9546-48
pGEX-6P-2 (25 µg)	28-9546-50
pGEX-6P-3 (25 µg)	28-9546-51
pGEX-2T (25 µg)	28-9546-53
pGEX-1λT EcoR/BAP (5 µg)	28-9546-56
pGEX 5′ Sequencing Primer 5′-d[GGG-CTGGCAAGCCACGTTTGGTG]-3′	27-1410-01
pGEX 3′ Sequencing Primer 5′-d [CCG-GGAGCTGCATGTGTCAGAGG]-3′	27-1411-01
E. coli BL21 1 vial	27-1542-01

GST purification products	**Code No.**
GST GraviTrap (10 columns)	28-9523-60
LabMate PD-10 Buffer Reservoir (50)	18-3216-03
GST Buffer Kit	28-9523-61
GST Bulk Kit	27-4570-01
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
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
Glutathione Sepharose 4B (10 ml)	17-0756-01
Glutathione Sepharose 4B (100 ml)	17-0756-05
Glutathione Sepharose 4B (300 ml)	17-0756-04

[1] Pack size available by specific customer order.

GST detection product	**Code No.**
GST Detection Module	27-4590-01
GST Detection Module (96-well format)	27-4592-01
Anti-GST Antibody	27-4577-01
ECL GST Western Blotting Detection Kit	RPN1237

Site-specific Proteases	**Code No.**
PreScission Protease (500 units)	27-0843-01
Thrombin (500 units)	27-0846-01
Factor Xa (400 units)	27-0849-01

Lysis kit	**Code No.**
Yeast Protein Extraction Buffer Kit	28-9440-45
Mammalian Protein Extraction Buffer	28-9412-79

Literature	**Code No.**
GST Gene Fusion System Handbook	18-1157-58
Recombinant Protein Purification Handbook	18-1142-75
Affinity Chromatography Handbook	18-1022-29

Legal

For your local office contact information, visit
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