GE Healthcare

pGEX-2TK GST Expression Vector

Product Specification Sheet

Code: 28-9546-46

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; *Not* I restriction endonuclease sites.

Protocols

Prepare fusion construct by inserting gene of interest into the multiple cloning site of pGEX-4T-3 using any one, or combination of unique restriction sites and transform into a host of choice such as *E. coli* BL21 (27-1542-01).

pGEX-2TK contains the recognition sequence for the catalytic subunit of cAMP-dependent heart muscle protein kinase (1), located between the GST domain and the multiple cloning site. Expressed proteins can be directly labeled using protein kinase and [γ-^{32}P] ATP and readily detected using standard radiometric or auto-radiographic techniques. pGEX-2TK is a derivative of pGEX-2T and fusion proteins can be cleaved with thrombin.

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.

[γ-^{32}P]ATP labelling of fusion proteins:

Reagents:

10X HMK Buffer: 200 mM Tris (pH 7.5), 1 M NaCl, 120 mM $MgCl_2$.

Bovine Heart Kinase: 10 U/µl in 40 mM dithiothreitol (kinase is available from Sigma Chemical Co. as a lyophilized powder, catalog number P-2645; make fresh, keep on ice).

[γ-^{32}P]ATP: 3000 Ci/mmole.

Protein Kinase Reaction Mixture: 15 µl 10X HMK Buffer, 5 µl bovine heart kinase solution, 5 µl [γ-^{32}P]ATP, 125 µl distilled H_2O. (Make immediately before use, keep on ice).

Stop Solution: 10 mM sodium phosphate (pH 8.0), 10 mM sodium pyrophosphate, 10 mM EDTA, 1 mg/ml BSA.

Elution Buffer: 50 mM Tris (pH 8.0), 5 mM reduced glutathione

NOTE: This protocol assumes that a 50% slurry of Glutathione Sepharose™ 4B (17-0756-01) was used to affinity purify the GST fusion protein in a batch method. The starting material for this protocol is the pellet of fusion-protein-loaded-GSH-Glutathione Sepharose 4B obtained from the final wash after binding of fusion protein.

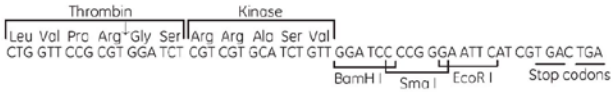
1. Wash the Glutathione Sepharose 4B GSH-Sepharose pellet using 5 ml of 1X HMK buffer per ml of Glutathione Sepharose 4B GSH-Sepharose slurry initially used to isolate the fusion protein. Centrifuge at 500 × g for 2 minutes to pellet the Glutathione Sepharose 4BGSH-Sepharose. Discard the supernatant.
2. Add 150 µl Protein Kinase Reaction Mixture per ml of slurry and vortex to mix. Incubate at 4°C for 30 minutes.
3. Add 5 ml of Stop Solution per ml of slurry. Centrifuge at 500 × g for 2 minutes. Properly discard the radioactive supernatant.
4. Wash the Glutathione Sepharose 4B GSH-Sepharose pellet with 5 ml of 1X PBS per ml of slurry. Centrifuge at 500 × g for 2 minutes. Properly discard the radioactive supernatant. Repeat this washing procedure for a total of 5 washes.
5. Elute the radiolabelled fusion protein using 1 ml of Elution Buffer per ml of slurry. Vortex to mix. Incubate at room temperature (25°C) for 5 minutes.
6. Centrifuge at 500 × g for 5 minutes. The supernatant contains the radiolabelled fusion protein.

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 (27-0846-01). 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 (2, 3).

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 (4). Increased solubilities can be obtained by lowering the growth temperature from 37°C to 28–30°C (5). 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. Kaelin, W. G., et al., *Cell* **70**, 351(1992).
2. Smith, D. B. and Johnson, K. S., *Gene* **67**, 31 (1988).
3. Eaton, D., et al., Biochemistry 25, 505 (1986).
4. Schein, C. H. and Noteborn, M. H. M., *Bio/Technology* **6**, 291 (1988).
5. 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-5X-1 (25 µg)	28-9545-53
pGEX-5X-2 (25 µg)	28-9545-54
pGEX-5X-3 (25 µg)	28-9545-55
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-3X (25 µg)	28-9546-54
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

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