Product Manual

GST ELISA Kit

Catalog Numbers

AKR-5185 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Glutathione S-transferase (GST) is a naturally-occurring 26 kDa protein found in eukaryotic cells. The gene from the parasitic helminth *Schistosoma japonicum* was used in the development of the GST affinity tag to aid in the purification of recombinant proteins. The 26 kDa GST moiety binds with high affinity to glutathione coupled to a matrix. This binding is reversible and the protein can be eluted under mild, non-denaturing conditions by the addition of reduced glutathione to the elution buffer. A specific protease site engineered between the GST moiety and the protein of interest allows removal of the GST moiety from the target recombinant protein. The GST can be removed from the sample by re-chromatography on a glutathione column, and the protein of interest purified to homogeneity by other techniques such as gel filtration or ion exchange.

Cell Biolabs' GST ELISA Kit is an enzyme immunoassay developed for detection and quantitation of GST or GST-tagged proteins in cell or tissue samples. The kit has detection sensitivity limit of 78 pg/mL GST. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and GST samples.

Assay Principle

The GST protein standards, and the unknown GST or GST-tagged protein samples, are first added to an anti-GST polyclonal antibody coated plate. After incubation, an FITC-conjugated anti-GST antibody is added, followed by an HRP-conjugated mouse anti-FITC antibody. The GST-tag protein content in unknown samples is determined by comparing with a standard curve that is prepared from GST protein standards.

Related Products

1. AKR-100: β-Galactosidase Staining Kit
2. AKR-110: Rapid GST Inclusion Body Solubilization and Renaturation Kit
3. AKR-121: GFP ELISA Kit
4. AKR-130: His Tag Protein ELISA Kit
5. AKR-5186: Streptavidin ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-GST Antibody Coated Plate (Part No. 51851B): One strip well 96-well plate.
2. FITC-Conjugated Anti-GST Antibody (Part No. 51852C): One 20 µL vial.
3. HRP-Conjugated Anti-FITC Monoclonal Antibody (Part No. 310811): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. GST Standard (Part No. 51853D): One 100 µL vial of 1 µg/mL recombinant GST (Met1-Lys218) in PBS containing BSA.

Materials Not Supplied

1. GST or GST Fusion Protein Sample: cell or tissue lysate
2. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the recombinant GST Standard at -20ºC and avoid freeze/thaw. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- FITC-Conjugated Anti-GST Antibody and HRP-Conjugated Anti-FITC Monoclonal Antibody: Immediately before use dilute the FITC-conjugated antibody 1:1000 and HRP-conjugated antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of GST Standard

Prepare a dilution series of recombinant GST standards in the concentration range of 0 ng/mL to 5 ng/mL in Assay Diluent (Table 1).

Standard Tubes	1 µg/mL Recombinant GST Standard (µL)	Assay Diluent (µL)	GST (pg/mL)
1	5	995	5000
2	500 of Tube #1	500	2500
3	500 of Tube #2	500	1250
4	500 of Tube #3	500	625
5	500 of Tube #4	500	313
6	500 of Tube #5	500	156
7	500 of Tube #6	500	78
8	0	500	0

Table 1. Preparation of samples for GST Standard Curve

Assay Protocol

1. Prepare cell or tissue lysates containing GST or GST-tagged protein.
2. Add 100 µL of GST sample or GST standard to the Anti-GST Antibody Coated Plate. Each GST sample, GST standard and blank should be assayed in duplicate.
3. Incubate at 37ºC for at least 2 hours or 4ºC overnight.
4. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted FITC-Conjugated Anti-GST Antibody to each well.
6. Incubate at room temperature for 2 hours on an orbital shaker.
7. Wash the strip wells 3 times according to step 4 above.
8. Add 100 µL of the diluted HRP-Conjugated Anti-FITC Monoclonal Antibody to all wells.
9. Incubate at room temperature for 1 hour on an orbital shaker.
10. Wash the strip wells 3 times according to step 4 above. Proceed immediately to the next step.
11. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

    Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
12. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
13. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical results with the GST ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

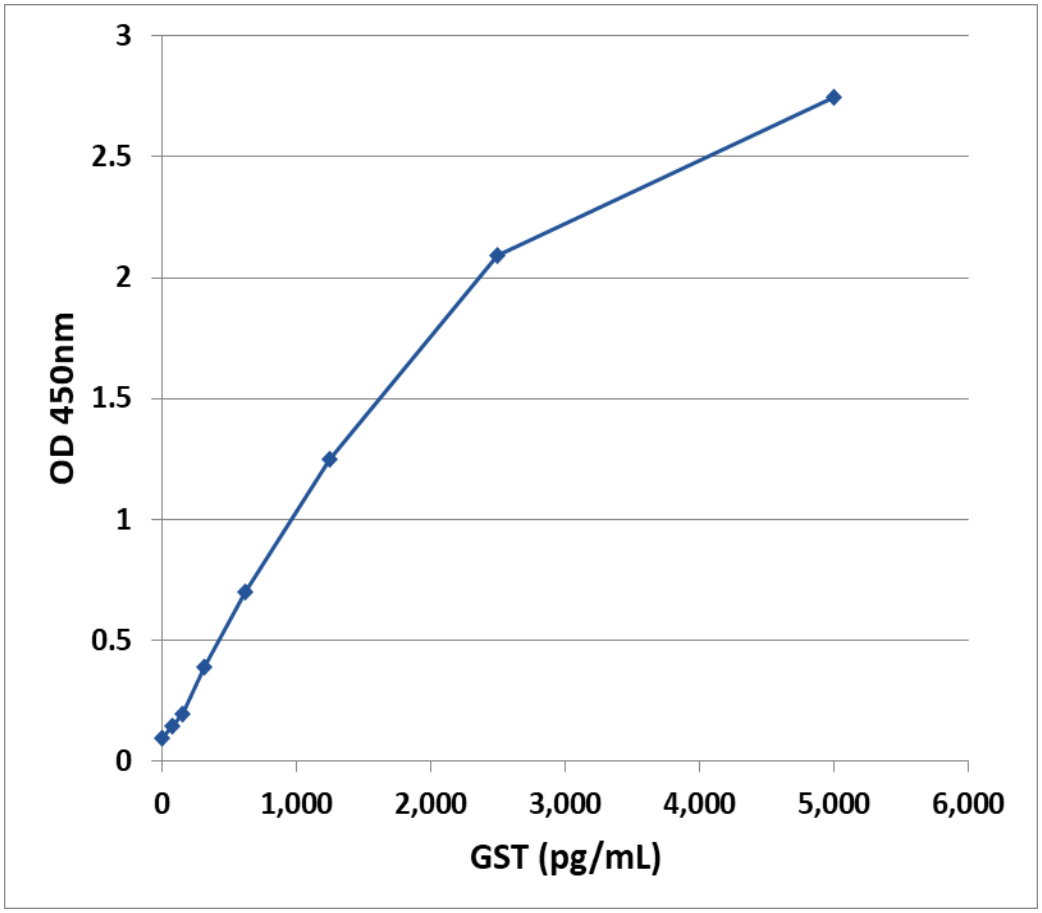

Figure 1: GST ELISA Standard Curve.

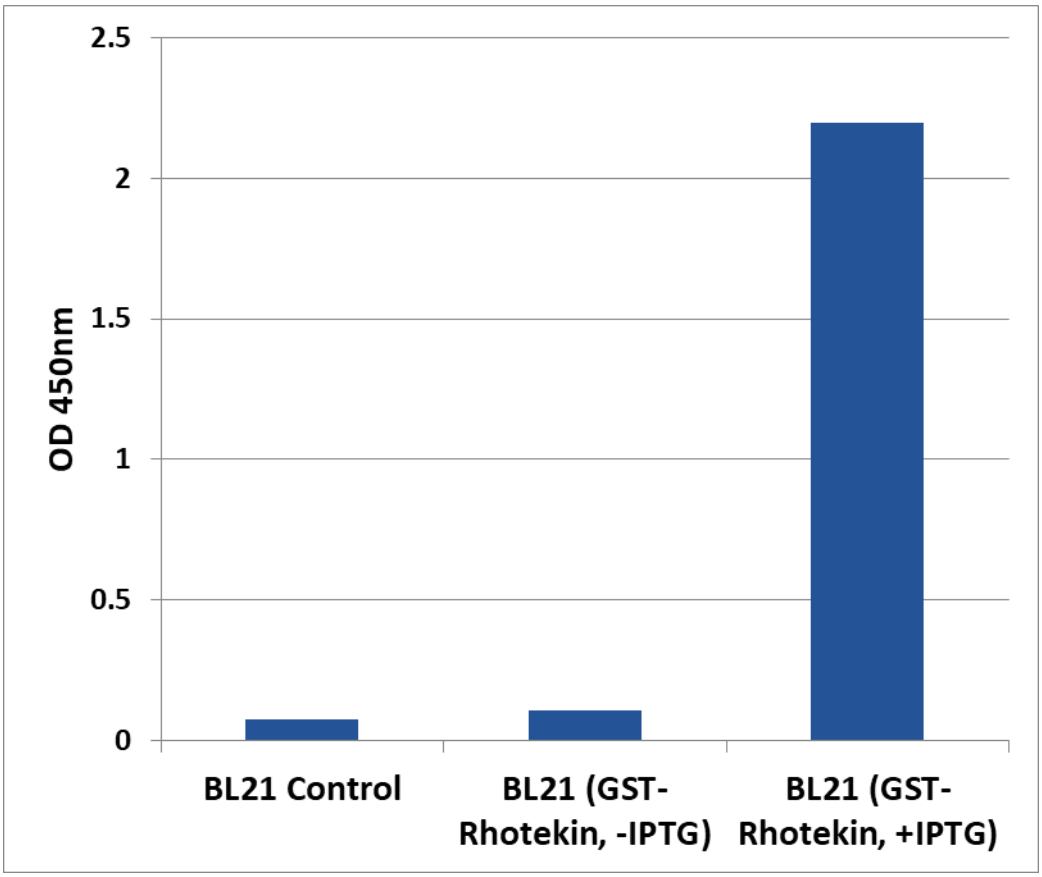

Figure 2: GST-Rhotekin in BL21 Bacterial Lysate. BL21 E.Coli lysate was first diluted to 0.2 mg/mL with bacterial sonication buffer (PBS/1% Triton X-100), then further dilute 1000 fold with Assay Diluent. Diluted E.Coli lysate was subjected to GST ELISA Kit according to the Assay Protocol.

References

1. Simons, P.C. and VanderJagt, D.L. (1977). *Anal Biochem* **82**:334-41.
2. Smith DB, Johnson KS. (1988) *Gene.* **67**:31–40.
3. Frangioni, J.V. and Neel, B.G. (1993). *Anal Biochem* **210**:179-87.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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