GST Tag ELISA Kit

12/18

(Catalog # E4690-100; 96 assays, Store kit at -20°C)

I. Introduction:

Glutathione S-transferase (GST) is a 26kDa protein and it is one of the most commonly-used tags for protein expression. Typically, it is expressed as part of fusion proteins to promote protein expression, solubility or stability. GST tagged proteins in E. coli or mammalian cell lysate can be easily purified by affinity chromatography on glutathione resin. Expression and detection of GST tagged proteins are frequently performed in academic and industry laboratories. BioVision's GST Tag ELISA Kit is a sandwich ELISA kit and it is an easy, fast, selective and sensitive method to detect GST and GST fusion proteins. Our detection kit offers ready-to-use reagents, and can detect as low as 45 ng/ml of GST Tag in approximately 3 hrs.

II. Applications:

In vitro, quantitative determination of GST or GST fusion proteins
Detection Range: 45 - 1440 ng/ml
Sensitivity: 45 ng/ml

III. Sample Type:

E. coli and mammalian cell lysates

IV. Kit Contents:

Components	E4690-100	Cap Code	Part Number
ELISA Microplate	8 X 12 Strips	--	E4690-100-1
GST Standard	1 vial	Yellow	E4690-100-2
HRP-conjugate stock	25 µl	Blue	E4690-100-3
Antibody	7 ml	NM	E4690-100-4
TMB substrate	10 ml	Amber	E4690-100-5
Stop Solution	10 ml	NM/Blue	E4690-100-6
Conjugate Buffer	7.5 ml	NM/Green	E4690-100-7
Wash Buffer (10X)	50 ml	NM	E4690-100-8
Plate Sealers	4	--	E4690-100-9

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 and 650 nm
- Clean eppendorf tubes for preparing standards and sample dilutions
- 1X PBS

VI. Storage and Handling:

The entire kit may be stored at -20°C for up to 12 months from the date of shipment. Opened kit is stable for 1 month at -20°C.

VII. Reagent and Standard Preparation:

Bring all buffers to room temperature before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- **Wash Buffer (10X)**: Bring bottle to room temperature. If crystals are present, warm up to room temperature and mix gently until the crystals are completely dissolved. Prepare 100 ml of 1X Wash Buffer by diluting 10 ml of Wash Buffer (10X) with 90 ml deionized water. The 1X solution can be stable at 4°C for one month.
- **HRP-conjugate**: Pipet 20 µl of HRP-conjugate into Conjugate Buffer (7.5 ml) bottle to prepare conjugate working solution. Vortex the conjugate solution bottle for a minute. The conjugate working solution is stable at 4°C for 2 months.
- **GST Standards**: Add 1.5 ml of deionized water into the vial to prepare 1440 ng/ml (S6). Perform 2-fold serial dilutions from S6 (e.g. 100 µl in 100 µl of water) to prepare S5 to S1 standards sequentially. S0 contains water only. Keep the prepared standards on ice during assay. The diluted standards are stable for 2-3 weeks at -20°C (avoid freeze-thaw cycles).

Standards	S0	S1	S2	S3	S4	S5	S6
Concentrations (ppb)	0	45	90	180	360	720	1440

VIII. Sample Preparation:

Notes: Always prepare a control without GST tagged protein expression (empty vector) to subtract background. The dilution factors are offered as below but users should determine final dilution rate depending on your sample concentration.

- **E. coli**

1. Spin 5 ml of E. coli cells (OD at 600 nm > 1.5) in a centrifugation tube at 10,000 x *g* at RT for 2 min.
2. Discard the medium and collect the pellet.
3. Add ~3 ml of PBS and vortex to disperse the pellet.
4. Lyse the cells by sonication for 2 min and then spin the cells at 10,000 x *g* and 4°C for 15 min.
5. Collect the supernatant and discard the pellet.
6. Dilute the supernatant 5 folds in 1X PBS (e.g. 100 µl in 400 µl of PBS).
7. Use 50 µl per well for the assay. (**Note**: Dilution factor: 5)

- **Mammalian Cells**

1. Centrifuge 2-3 ml of mammalian cells (10^5 – 10^6 cells/ml) at 5,000 x *g* and RT for 2 min.
2. Collect the pellet and discard the supernatant.

3. Add 0.5-1 ml of 1X PBS into the tube and vortex to disperse the cell pellet.
4. Lyse the cells by sonication for 2 min on ice and then spin the cells at 10,000 x *g* and 4°C for 10 min.
5. Collect the supernatant.
6. Dilute the supernatant 10 folds (e.g. 100 µl in 900 µl of deionized water).
7. Use 50 µl per well for the assay. (**Note**: Dilution factor: 10)

IX. GST-Tag ELISA Assay Protocol:

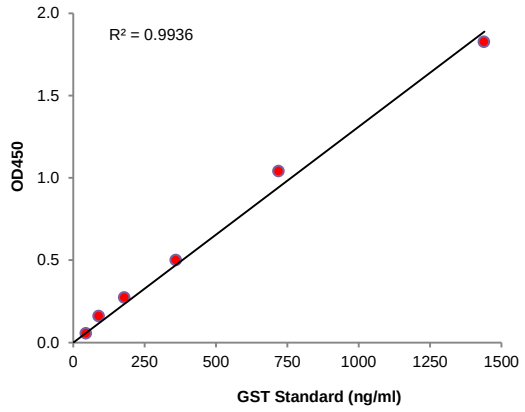
Notes: It is recommended that all standards and samples should be run at least in duplicate. Standard curves must be run each time an assay is performed.

1. Prepare all reagents, standards and samples as sections VII and VIII respectively.
2. Add 50 µl of Standards or Samples per well. Then add 50 µl conjugate working solution and 50 µl Antibody to above wells.
3. Cover the plate with a plate sealer and mix well. Incubate the plate at room temperature (25°C) for 120 min.
4. Aspirate all reagents and wash each well 5 times: add 250 µl of 1X Wash Buffer and incubate for 30 seconds. Remove 1X Wash buffer completely before the next wash - it is essential for accurate results. Repeat this step 4 more times.
5. Add 100 µl of TMB Substrate to each well. Tap or shake the plate to ensure complete mixing.
6. Check the OD at 650 nm for the well containing highest GST standard (S6). When its reading is between 0.8 and 1.0 (usually between 10-30 min after adding the TMB Substrate), add 50 µl of Stop Solution and gently tap the plate to ensure thorough mixing.
7. Measure the OD at 450 nm for the standards and samples immediately.

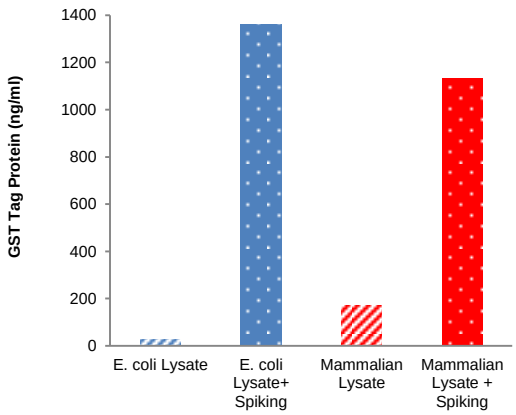
X. Calculation:

The Standard Curve is done by plotting OD 450 nm of each standard solution (Y) vs. the respective concentration of the standard solution (X). The concentration of GST protein in each sample (ng/ml) can be interpolated from the standard curve. Apply the coefficient that represents the ratio between the fusion protein MW and GST MW (X kD/26 kD) to the concentration of GST in the sample (where X is the MW of the fusion protein including the GST polypeptide). This will give you the concentration of the fusion protein (ng/mL). If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

A.



B.



Figures. **A.** Standard curve for GST Tag ELSIA Kit (*This standard curve is for demonstration only. A standard curve must be run with each assay*). **B.** Spike recovery experiment: E. coli and mammalian cell lysates were spiked with 1440 ng/ml of GST tagged protein and showed 80-95% of recovery.

XI. RELATED PRODUCTS:

His-Tag Protein ELISA Kit (Cat. No. E4550-100)
Gentamicin (serum/urine) ELISA Kit (Cat. No. K4315-100)
Ampicillin ELISA Kit (Cat. No. E4350-100)
Enrofloxacin (ENR) ELISA Kit (Cat. No. E4277-100)
Fluoroquinolones ELISA Kit (Cat. No. K4205-100)
Vancomycin ELISA Kit (Cat. No. E4605-100)
Folic Acid ELISA Kit (Cat. No. E4523-100)
Kanamycin ELISA Kit (Cat. No. K4210-100)
Quinolone ELISA Kit (Cat. No. E4530-100)
Caffeine ELISA Kit (Cat. No. E4558-100)

FOR RESEARCH USE ONLY! Not to be used on humans.