GST tag ELISA Detection Kit

Technical Manual No. TM0521 **Version 01262011**

I. DESCRIPTION

GenScript GST tag ELISA Detection Kit is designed to quantitate glutathione-S-transferase (GST) or GST-fusion protein in samples such as cell lysate using a proprietary ELISA procedure. This kit allows user to detect and quantitate GST or GST-fusion protein in just 60 minutes. The assay uses a pair of monoclonal antibodies specific to GST. Both capture and detection antibodies are Anti-GST monoclonal antibodies. This kit provides a rapid and reliable method to detect GST-fusion protein.

II. KIT CONTENTS

The kit contains all necessary reagents and buffers for performing 5 x 96 tests.

Warning: Wear gloves when handling the reagents. Some of them are corrosive!

Kit Components	**L00411 For 5 Plates**
Pre-coated microwell plate (12 wells x 8 strips)	5
Dilution solution	100 ml
HRP conjugated GST monoclonal antibody	60 ml
GST protein stock (1.0 mg/ml)	40 µl
20X Wash solution	55 ml
One-Component TMB Substrate	55 ml
Stop solution	55 ml
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III. RELATED PRODUCTS

- One-component TMB substrate M00078
- Stopping Solution M01017
- 20X Washing Solution M01016

IV. KEY FEATURES AND APPLICATIONS

- Time saving: This kit allows user to quantitate GST or GST-fusion protein in just 60 minutes.
- Highly producible results

Application:

- Quantitate GST or GST-fusion proteins.
- Check GST or GST-fusion protein expression.
- Screen GST-fusion protein expression for expression optimization.

V. STORAGE

- The unopened kit is stable for at least 6 months stored at 2-8°C.
- The opened kit may be stored for up to 1 month at 2-8°C.
- Use only before expiration date.
- Do not freeze the kit.

VI. ELISA PROTOCOL

This procedure is optimized for one 96-well plate. The volumes of the reagents can be scaled up or down according to the numbers of the plates used.

Reagent Preparation:

Note: All reagents must be allowed to equilibrate to room temperature prior to use.

Wash buffer: Dilute 10 ml of 20X Washing Solution with 180 ml of distilled or deionized water to make 200 ml of 1X Washing Solution. If any precipitate forms in the 20X wash solution during storage, incubate the bottle in water bath (up to 50°C) with occasional mixing until all the precipitate disappears.

GST standard: Dilute GST protein stock with dilution solution to make following GST standards: 100 ng/ml, 50 ng/ml, 25 ng/ml, 12.5 ng/ml and 6.25 ng/ml, 3.125ng/ml, 0.00ng/ml, respectively. Carefully calculate and make enough standards for assay in duplicate or triplicate.

Assay procedure:

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1. Add 100 µl of prepared standards, samples, blanks (without GST protein) to different wells as shown in suggested plate scheme below and incubate the plate for 30 min at 37°C. After incubation, remove and discard the solutions. Wash the wells 3 times with 250 µl of 1x Wash Solution.
2. Add 100 µl of HRP conjugated GST monoclonal antibody to all wells and incubate the plate for 15 min at 37°C. Wash the wells 5 times with 250 µl of 1X Wash Solution.
3. Add 100 µl of One-Solution Microwell TMB substrate to each well and incubate at room temperature for 10 minutes.
4. Add 100 µl/well of Stop Solution to stop the enzyme reaction (the blue reaction mixture will turn yellow). Measure absorbance at 450nm.
5. Generate a standard curve by plotting the average absorbance on the vertical axis versus the corresponding GST standard concentration on the horizontal axis. The data can be linearized by using a log/log plot and regression analysis can be applied to the log transformation. The amount of GST in each sample is determined by extrapolating OD values to GST concentrations using the standard curve.

Suggested Plate Scheme

	Standard (ng/ml)		Sample wells									
	1	2	3	4	5	6	7	8	9	10	11	12
A	100	100										
B	50	50										
C	25	25										
D	12.5	12.5										
E	6.25	6.25										
F	3.125	3.125										
G	0.00	0.00										
F												

VII. Typical assay data

Conc. Of antigen（ ng/ml）	**GST Kit of GenScript**		
	OD1	OD2	Average
100	2.555	2.600	2.5775
50	1.461	1.372	1.4165
25	0.874	0.847	0.8605
12.5	0.507	0.480	0.4935
6.25	0.322	0.321	0.3215
3.125	0.230	0.219	0.2245
0	0.138	0.132	0.1350

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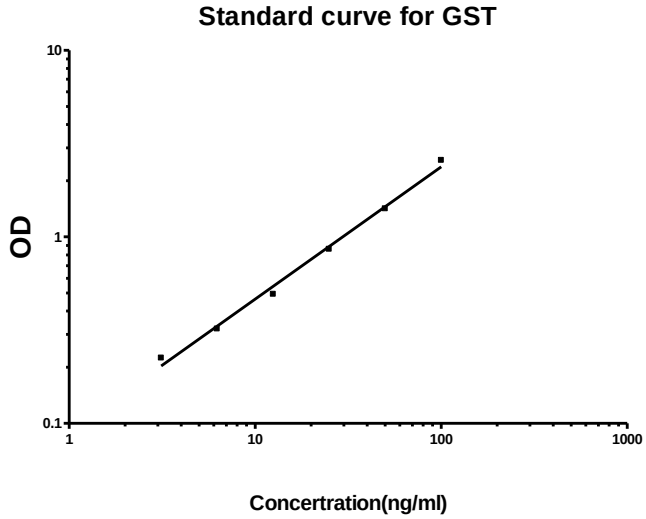

VIII. TROUBLESHOOTING

Use the table below to solve and avoid common problems.

Problem	Probable Cause	Solution
The OD_{450} is too low.	Too little GST is in the sample.	Don't dilute the samples.
	There is not much GST or GST-fusion protein in samples.	Improve protein expression procedure.
	The kit is not stored properly.	The kit should be stored at 4°C.
OD_{450} of blank or negative is too high.	Blank or negative is contaminated.	Avoid contamination of kit reagents and work at a clean place.
	There is carryover of unbound reagents, especially GST ELISA solution.	Make sure enough wash solution is added to each well.
	The wavelength of the reader is set at the wrong wavelength.	Check the wavelength setting.

IX. ORDERING INFORMATION

GST tag ELISA Detection Kit: Cat. No. L00411 for GST or GST-fusion protein quantification.

Patent Pending.

For Research Use Only.

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