

One-Step ELISA™ GST Detection Kit

Technical Manual No. TM0240

Version 10302009

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I. DESCRIPTION

The GenScript One-Step ELISA™ GST Detection Kit is designed to quantitate glutathione-S-transferase (GST) or GST-fusion protein in samples such as cell lysate using a proprietary one-step ELISA procedure. This kit allows user to detect and quantitate GST or GST-fusion protein in just 35 minutes. The One-Step ELISA™ procedure is contrasted with a classical five-hour sandwich ELISA procedure at right (figure 1).

Incubate the pre-coated and pre-blocked plate with the sample and the ELISA solution included in the kit for 20 minutes, wash the plates 5 times, and the plate can then be developed with the One-Solution Microwell TMB solution included in the kit.

The kit contains all necessary reagents and buffers for performing ELISA using five 96-well plates. No secondary antibody is needed.

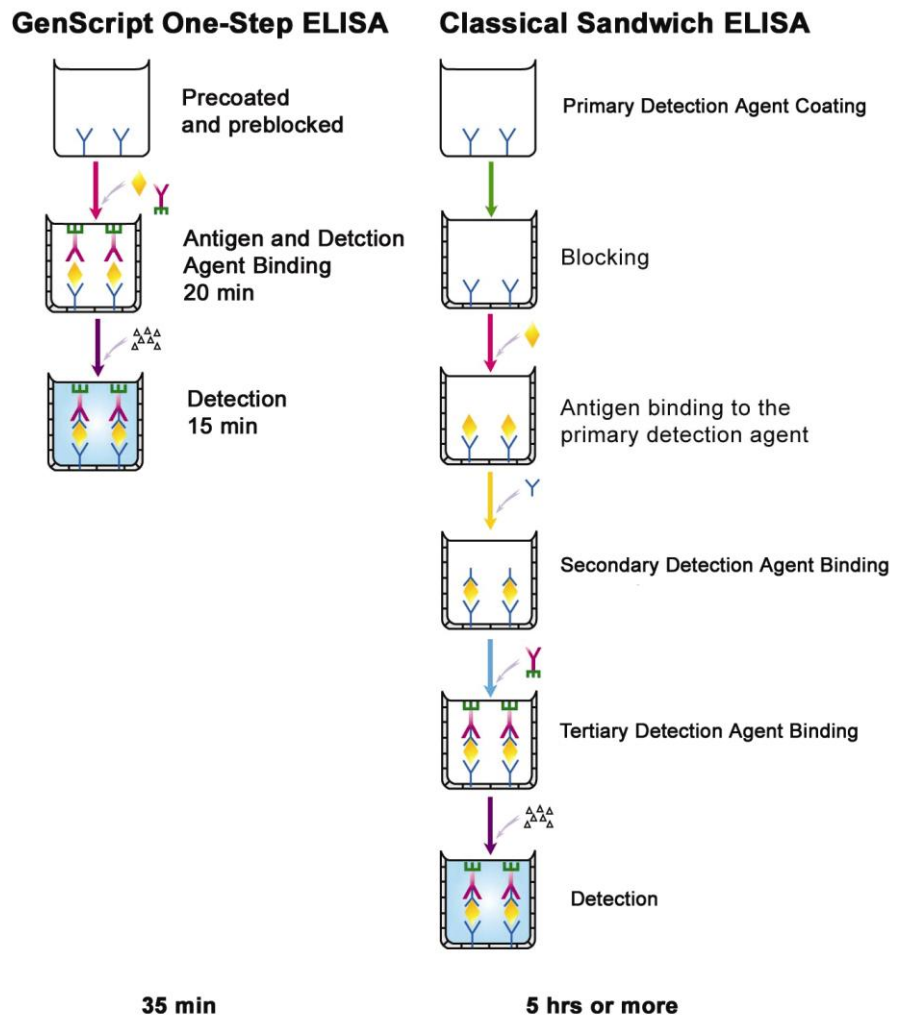


Figure 1. Overview of Sandwich ELISA Procedures

II. KIT CONTENTS

The kit contains enough reagents for five 96-well plate ELISA assays.

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

| Kit Components | L00254 For 5 Plates |
|--|------------------------|
| Pre-coated plate (12 strips per plate) | 5 |
| Dilution solution | 100 ml |
| GST ELISA solution | 30 ml |
| GST standard protein (0.7 mg/ml) | 40 μ l |
| 20X Wash solution | 55 ml |
| One-Solution Microwell TMB | 55 ml |
| Stop solution | 55 ml |
| Protocol | 1 |

III. RELATED PRODUCTS

- One-Solution Microwell TMB M00078
- Stop Solution M01017
- 20X Wash Solution M01016

IV. KEY FEATURES AND APPLICATIONS

- ◆ Quick: This kit allows user to quantitate GST or GST-fusion protein in just 35 minutes.
- ◆ Reproducible results: The kit produces highly reproducible results.

The kit can be used for:

- ◆ Detecting GST or GST-fusion proteins.
- ◆ Checking GST or GST-fusion protein expression.
- ◆ Screening GST-fusion protein expression for expression optimization.

V. STORAGE

Store the kit at 4°C. It will remain stable for six months. **Do not freeze the kit or any of its components.**

VI. ONE-STEP 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.

Before use, prepare the following:

1. Dilute 10 ml of 20X wash solution with 180 ml of distilled or filtered water to make 200 ml of 1X wash solution. If any precipitate forms in the 20X wash solution during storage, incubate the bottle in warm or hot water bath (up to 50°C) with occasional mixing until all the precipitate disappears. Dilute the buffer with ddH₂O to 1X and store it at 4°C.

- Dilute the GST standard protein with dilution solution to make a dilution series of standard solutions with concentrations of 500 ng/ml, 167 ng/ml, 55.6 ng/ml, 18.5 ng/ml, 6.17 ng/ml and 2.05 ng/ml, respectively. Standard dilution series should be run in duplicate or triplicate.
- Dilute the samples (such as cell lysate) with dilution solution to make a dilution series. Standard dilution series should be run in duplicate or triplicate. Assays, including samples and controls, should be performed in duplicate or triplicate.

One-Step ELISA™ procedure:

- Adding samples and incubation.** Add 50 µl of diluted samples, blanks (dilution solution only) and negative controls (NC: such as BSA in Dilution Solution) to different wells as shown in suggested plate scheme below. Then add 50 µl of GST ELISA Solution to each well and incubate the plate for 20 min at room temperature. After incubation, remove and discard the solutions. Wash the wells 5 times with 250 µl of 1x Wash Solution.
- Development with TMB.** Add 100 µl of One-Solution Microwell TMB substrate to each well and incubate at room temperature for 15 minutes. Shake gently for best results.
- Stop reaction.** Add 100 µl/well of Stop Solution to stop the enzyme reaction (the blue reaction mixture will turn yellow). Measure absorbance at 450nm. The color should be read within 30 minutes.
- Concentration determination.** 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

- Using one strip for testing or detection.**

| Standard (ng/ml) | | | | | Sample wells | | |
|------------------|------|------|----|-----|--------------|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 500 | 55.6 | 6.17 | NC | Blk | | | |

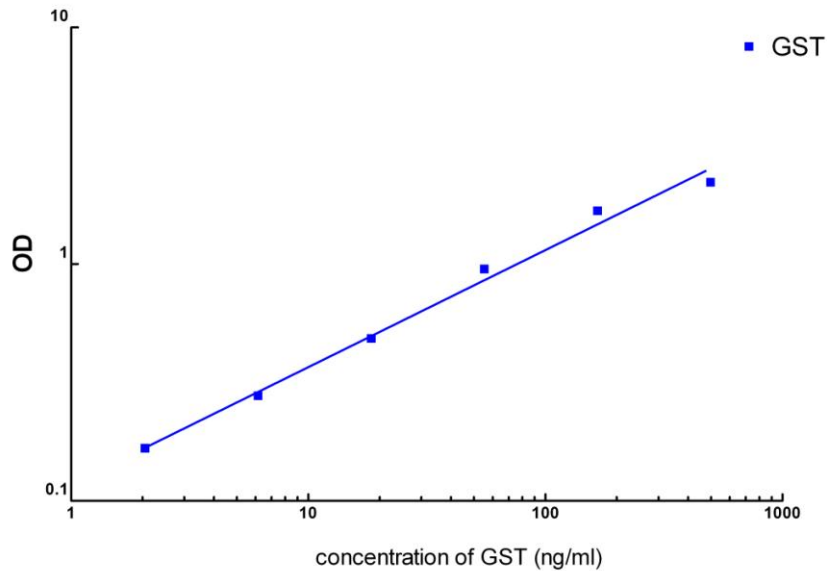
- Using one plate for quantitation.**

| Standard (ng/ml) | | Sample wells | | | | | | | | | | |
|------------------|------|--------------|---|---|---|---|---|---|---|----|----|----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 500 | 500 | | | | | | | | | | |
| B | 167 | 167 | | | | | | | | | | |
| C | 55.6 | 55.6 | | | | | | | | | | |
| D | 18.5 | 18.5 | | | | | | | | | | |
| E | 6.17 | 6.17 | | | | | | | | | | |
| F | 2.05 | 2.05 | | | | | | | | | | |
| G | NC | NC | | | | | | | | | | |
| H | Blk | Blk | | | | | | | | | | |

VII. EXAMPLES

| Conc. of antigen | GST kit of GenScript | | |
|------------------|----------------------|-------|---------|
| | OD1 | OD2 | Average |
| 500 | 2.222 | 2.204 | 2.213 |
| 167 | 1.629 | 1.718 | 1.6735 |
| 55.6 | 0.935 | 0.966 | 0.9505 |
| 18.5 | 0.463 | 0.504 | 0.4835 |
| 6.17 | 0.267 | 0.286 | 0.2765 |
| 2.05 | 0.167 | 0.165 | 0.166 |
| 0 | 0.111 | 0.105 | 0.108 |

Standard curves of one-step GST Elisa Detection Kit



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

One-Step ELISA™ GST Detection Kit:

L00254 for GST or GST-fusion quantitation.

Patent Pending.

For Research Use Only.

Limited Use Label license: This product may be the subject of one or more patents filed by GenScript Corporation. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer may not sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for any commercial purposes. For commercial use, please contact GenScript at info@genscript.com.

GenScript Corporation
 120 Centennial Ave., Piscataway, NJ 08854
 Tel: 732-885-9188, 732-885-9688
 Fax: 732-210-0262, 732-885-5878
 Email: info@genscript.com
 Web: <http://www.genscript.com>