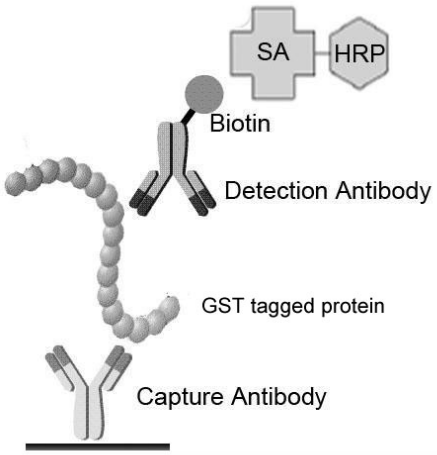
GST Tag ELISA Detection Kit **Cat. No. L00411**

Technical Manual No. TM0521 **Version 05172012**

Genscript GST Tag ELISA Detection Kit is used for the detection of GST tagged protein in samples.



The operator should read the technical manual carefully before using this product.
For research use only. Not for use in diagnostic procedures.

Contents

I. Description

Glutathione S-transferase (GST) Tag is engineered to create the GST gene fusion system, a system used to purify and detect proteins of interest. The GST Tag is composed of 220 amino acids, with a molecular weight of 26 kD and GST tagged proteins is expressed in high levels in bacteria expression system and the fusion protein is purified in a single step by affinity chromatography using glutathione agarose. With site-specific proteases, such as thrombin or factor Xa, the GST portion can easily be removed from GST tagged protein. GST tag antibody is a universal tool for detection of GST tagged protein.

GenScript GST Tag ELISA Kit was developed for three purposes, to identify GST tagged protein, monitoring the protein expression and for screening the expression optimization of the protein. It can also detect residual GST in the GST tag cleavage during protein purification. However it is possible that improper folding of GST or the presence of a fusion partner may prevent GST binding with the GST monoclonal antibodies in this kit.

The kit detects GST tagged protein in a sandwich immunoassay. It utilizes two clones of GST monoclonal antibodies that bind to different epitopes of the GST tag protein at the same time. The GST monoclonal antibody, pre-coated on the plate, is used to capture GST tagged protein and the biotin conjugated GST monoclonal antibody (Biotin-GST Mab) is added to interact with GST tagged protein bound on the plate. Streptavidin-HRP is added to interact with Biotin-GST Mab and the final step involves the addition of TMB Substrate that will react with HRP to give product with an absorbance at 450 nm. The quantity of GST tagged protein in sample is determined by comparisons with the absorbance of that of a known GST tagged protein standard curve.

II. Key Features

- High Sensitivity: 1 ng/ml GST tagged protein
- Broad Detection Range: 3.125~100 ng/ml
- Application: Identifying GST tagged protein
  Checking GST tagged protein expression and screening the expression optimization
  Detecting residual GST in the GST tag cleavage during protein purification
  Rapid quantification
- Feasible Operation: Provide all reagents required for test
  Complete the test in an hour

III. Kit Contents

The kit provides all components necessary for GST tagged protein tests. Sufficient material is provided in this kit to perform tests of one plate (96 well).

Components	Quantity	Part.No
GST Capture Plate	1 plate (8 wells x 12 strips)	411-80
Sample Dilution Buffer	30 ml	411-60
Biotin-GST Mab Stock	200 µl	411-20
Antibody Dilution Buffer	15 ml	411-90
Streptavidin-HRP	12 ml	411-30
GST Protein Stock (1.0 mg/ml)	10 µl	411-10
20 X Wash Solution	40 ml	411-70
TMB Substrate	12 ml	411-40
Stop Solution	6 ml	411-50
Adhesive Plate Cover	2 pieces	N/A
Kit Manual	1	N/A

IV. Storage

The unopened kit is stable for at least 12 months if stored at 2-8°C and the opened kit may be stable for up to 1 month at 2-8°C. **Do not freeze the kit**.

V. Reagents/Equipments Required But Not Supplied

Microplate reader capable of measuring absorbance at 450 nm

Automated microplate washer

Deionized or distilled water

Graduated cylinder to prepare Wash Solution

Plastic container to prepare Wash Solution

Tubes to prepare standard dilutions and to aliquot samples

Precision pipettes to deliver 10 µl, 100 µl, 200 µl and 1000 µl content

10 µl, 100 µl, 200 µl and 1000 µl pipette tips

Multichannel pipettor

Disposable reagent reservoirs

Tower paper

Laboratory timer

Refrigeratory to store samples and kit components

VI. Instruction for Use

Reagent Preparation

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

Wash Solution

Dilute *20 X Wash Solution* with distilled or deionized water by 1:20.

For example, dilute 10 ml of *20 X Washing Solution* with 190 ml of distilled or deionized water to make 200 ml of *Wash Solution.*

Note: If any precipitate forms in the 20 X Wash Solution during storage, incubate the bottle in water bath (up to 50 °C) with occasional mixing until all the precipitate disappears.

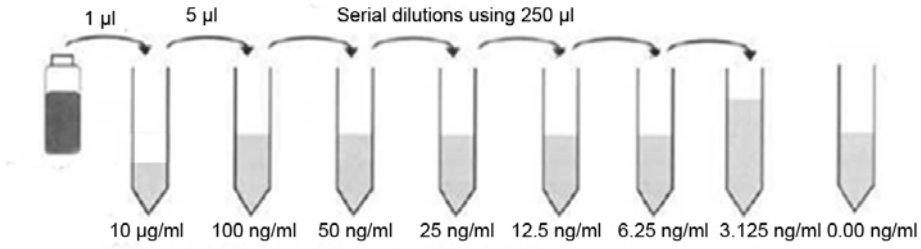
Biotin-GST Mab

Dilute Biotin-GST Mab Stock with *Antibody Dilution Buffer* by 1:40.

GST Standards

Prepare solutions for the GST standard curve

1. Label eight tubes, one for each standard curve point: '10 µg/ml', '100 ng/ml', '50 ng/ml', '25 ng/ml', '12.5 ng/ml', '6.25 ng/ml', '3.125 ng/ml' and '0 ng/ml'.
2. Pipette 1 µl of *GST Protein Stock* (1.0 mg/ml) into the 1.5 ml vial containing 99 µl of *Sample Dilution Buffer* to make 1*0 µg/ml GST solution*.
3. Pipette 495 µl of *Sample Dilution Buffer* into the first tube (100 ng/ml) and 250 µl of *Sample Dilution Buffer* into the following tubes.
4. Pipette 5 µl of *10 µg/ml GST solution* into the first tube (100 ng/ml) and mix.
5. Pipette 250 µl of *100 ng/ml GST standard solution* into the second tube (50 ng/ml) and mix.
6. Repeat the serial dilutions (using 250 µl) five times to complete the standard curve points. These concentrations should be 100, 50, 25, 12.5, 6.25, 3.125 and 0 ng/ml.



Sample Preparation

When preparing samples for the assay several important issues should be considered.

1. Preliminary experiments should be performed to optimize sample dilution. Dilute samples with Sample Dilution Buffer*, for example,* by 1:5, 1:10, 1:20 and 1:40.
2. Neutral pH is required for the assay. Samples should not contain any particles. Filter the sample or

centrifuge if needed to remove the insoluble material.

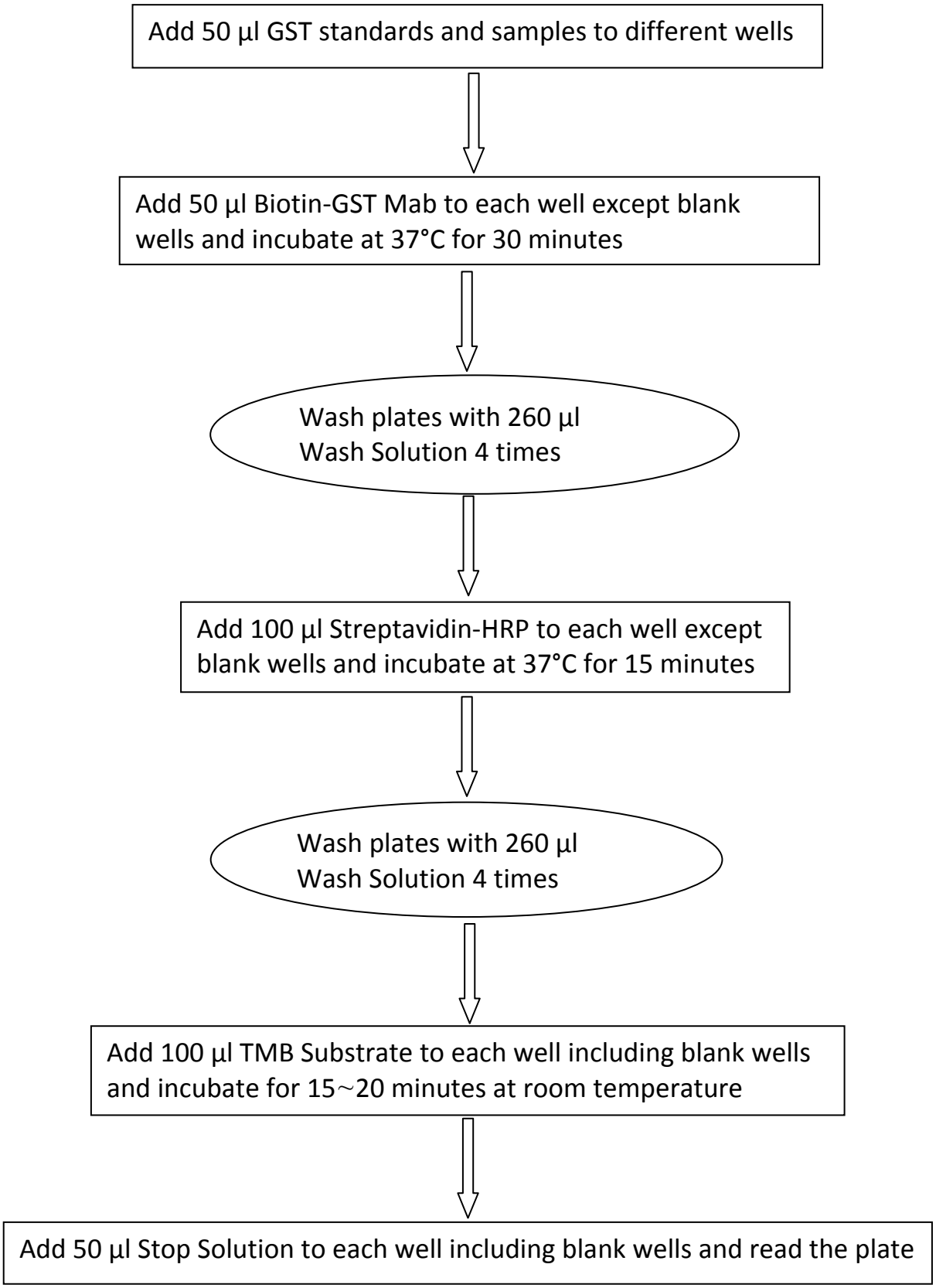
Setup of GST Standards and serial of test sample Dilutions on Microtiter Plate

	Standard (ng/ml)		Diluted Samples									
	Duplicate 1	Duplicate 2	3	4	5	6	7	8	9	10	11	12
A	100	100	1:5	1:5								
B	50	50	1:10	1:10								
C	25	25	1:20	1:20								
D	12.5	12.5	1:40	1:40								
E	6.25	6.25										
F	3.125	3.125										
G	0.00	0.00										
H	Plate Blank											

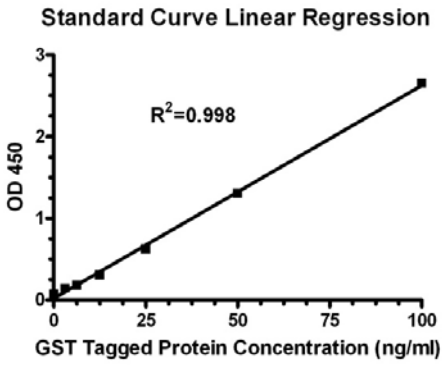
Assay Procedure

1. Add 50 µl of prepared standards and test samples into different wells except blank wells as shown in suggested plate scheme above.
2. Add 50 µl of prepared *Biotin-GST Mab* to all wells except blank wells.
3. Cover the Plate with *Adhesive Plate Cover* and incubate the plate for 30 minutes at 37°C.
4. Move *Adhesive Plate Cover* and remove the solution from the wells.
5. Wash the wells with 260 µl of *Wash Solution* for four times.
6. Invert the plate and pound it vigorously on clean towels to remove excess liquid in the wells.
7. Add 100 µl of *Streptavidin-HRP* to all wells except blank wells.
8. Cover the Plate with *Adhesive Plate Cover* and incubate the plate for 15 minutes at 37°C.
9. Move *Adhesive Plate Cover,* remove to solution from the wells and wash the wells with 260 µl of *Wash Solution for* four times.
10. Add 100 µl of *TMB Substrate* to each well including blank wells and incubate for 15~20 minutes at room temperature.
11. Add 50 µl of *Stop Solution* to each well including blank wells to stop the reaction.
    Note: the blue reaction mixture will turn yellow.
12. Read absorbance of each tested well on a Microplate reader using 450 nm as the primary wave length.
13. 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 linear regression analysis.
14. The amount of GST in each sample is determined by extrapolating absorbance values to the GST concentrations using the standard curve.

VII. Assay Procedure Summary



VIII. Typical Assay Data



Conc. of GST (ng/ml)	Conc. of GST (pmol/ml)	OD 450			
		Duplicate 1	Duplicate 2	Average	Adjusted Average
100	3.846	2.682	2.624	2.653	2.603
50	1.923	1.324	1.280	1.302	1.252
25	0.962	0.624	0.618	0.621	0.571
12.5	0.481	0.310	0.289	0.300	0.250
6.25	0.240	0.177	0.177	0.177	0.127
3.125	0.120	0.136	0.146	0.141	0.091
0	0	0.064	0.067	0.066	0.016
Blank		0.051	0.048	0.050	

The standard curve was provided as an example only. It should be prepared each time an assay is performed.

IX. Precision

Intra-assay Precision: CV<5%

Inter-assay Precision: CV<10%

X. Recovery

The added spike and recovery should be within allowable limit 85% to 115%.

XI. Troubleshooting

Problem	Probable Cause	Solution
Poor Precision	Wells were not washed or aspirated properly	Make sure the wash apparatus work properly and wells are dry after aspiration
	Wells have been scratched with pipette tip or washing needles.	Dispense and aspirate solution into and out of wells with caution
	Particulates were found in the samples	Remove any particulates by centrifugation prior to the assay
	Wells were not washed or aspirated properly	Make sure the wash apparatus work properly and wells are dry after aspiration
	Pipetting error	Check pipette calibration and repeat assay
	Components were used from other lots or sources	Never substitute any components from another kit
	Components were not brought to room temperature prior to assay.	Repeat assay with components that have been equilibrated to room temperature
Weak/No Signal	TMB Substrate were not added or were added at the wrong time	Follow the Manual to add the Substrate
	Detection Reagent was not added, or was added at the wrong time	Follow the manual to repeat the assay
	TMB Substrate has been contaminated	Use new TMB Substrate
	Did not add the proper volumes of reagents	Repeat assay with the required volumes in manual
	Did not incubate the plate for proper time or temperature	Follow the manual to repeat assay
	Did not read the plate immediately after Stop Solution was added	Read the plate within 30 minutes
High background	Plate was not washed properly	Make sure the wash apparatus is functioning properly. Make sure all wash buffers is removed before adding substrate
	TMB Substrate has been contaminated	Use new TMB Substrate with same Lot
	Evaporation of wells during incubations	Perform incubation steps with Adhesive Plate Cover in repeat assay
	Incorrect incubation times and/or temperatures	Follow the manual to repeat the assay
	TMB Substrate exposed to light	Use new TMB Substrate

XII. Related Products

- THE™ GST Antibody, mAb, Mouse A00865
- THETM GST Antibody [HRP], mAb, Mouse A00866
- THETM GST Antibody [Biotin], mAb, Mouse A00867
- Protein A ELISA Kit L00430
- One-Component TMB Substrate M00078
- Stop Solution M01017
- 20 X Wash Solution M01016

XIII. Plate Layout

Use this plate layout to record standards and samples assayed.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Notes:

Use this plate layout to record standards and samples assayed.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
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Notes: