

ELISA Assay for Glutathione S-Transferase Alpha

For Research Use Only

INTRODUCTION

Glutathione S-Transferase (GST) has multiple isoforms. This assay is specific for Glutathione S-Transferase Alpha (GSTA) and is not known to cross react with the mu, pi, or theta variants. GSTA is a common biomarker for hepatocellular damage.¹ It also conjugates GSH to 4-hydroxynonenal, a product of lipid peroxidation² and is an important player in cellular antioxidant defense mechanisms.³

PRINCIPLES OF PROCEDURE

This is a standard sandwich enzyme-linked immunosorbent assay (ELISA). The plate is pre-coated with anti-GSTA and blocked, ready for the addition of samples and standards. The assay should take approximately 3 hours to run, plus any required sample preparation time.

MATERIALS PROVIDED

Component	Description	Volume	Storage	Cat no.
Anti-GSTA Plate	96-well microplate coated and blocked	1 plate	4°C	GS41a
Assay Buffer	Buffer used to dilute samples and reagents	100 mL	4°C	GS41b
10x Wash Buffer	Buffer used to wash the plate	30 mL	4°C	GS41c
GST Alpha Standard	10 µg/mL GSTA	20 µL	4°C	GS41d
Detection Antibody	Anti-Human-GSTA	130 µL	4°C	GS41e
HRP-Conjugate	Streptavidin-HRP conjugate	130 µL	4°C	GS41f
TMB Substrate	Stabilized TMB color reagent	20 mL	4°C	GS41g

MATERIALS NEEDED BUT NOT PROVIDED

1. Microplate reader with 450 nm filter
2. Adjustable micropipettes and tips
3. 3N Sulfuric Acid (H₂SO₄)
4. Deionized Water (dH₂O)

STORAGE

1. Store the components of this kit at the temperatures specified on the labels.
2. Unopened reagents are stable until the indicated kit expiration date.

WARNINGS AND PRECAUTIONS

1. Use aseptic technique when opening and dispensing reagents.
2. This kit is designed to work properly as provided and instructed. Additions, deletions or substitutions to the procedure or reagents are not recommended, as they may be detrimental to the assay.

PROCEDURAL NOTES

1. Reagents can be used immediately upon removal from refrigeration.
 2. To minimize errors in absorbance measurements due to handling, wipe the exterior bottom of the microplate wells with a lint-free paper towel prior to inserting into the plate reader.
 3. Do not save excess or diluted reagents for future use.
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SAMPLE STORAGE

Samples should be stored at -80°C and thawed just prior to use. Avoid repeated freeze/thaw cycles for best results. This assay was developed and validated with human serum samples, however it does cross-react with rat.

SAMPLE PREPARATION

It is recommended to do multiple sample dilutions to ensure that the concentration falls within the accepted range for the assay. Samples should be assayed neat or diluted 1:2 in Assay Buffer.

REAGENT PREPARATION

1. **GST Alpha Standard:** Immediately prior to use, dilute 1:1000 by adding 10 μL of Standard to 10 mL of Assay Buffer, giving a final concentration of 10 ng/mL.
 2. **10x Wash Buffer:** Dilute the wash buffer 1:10 by adding 30 mL of 10x Wash Buffer to 270 mL of dH_2O .
 3. **Detection Antibody:** Immediately prior to use, dilute 1:100 by adding 120 μL of Detection Antibody to 12 mL of Assay Buffer.
 4. **HRP-Conjugate:** Immediately prior to use, dilute 1:200 by adding 60 μL of HRP-Conjugate to 12 mL of Assay Buffer.
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STANDARD CURVE PREPARATION

Set up for the standard curve by labeling dilution tubes and dispensing the indicated volumes of Assay Buffer and 10 ng/mL Standard Stock Solution according to Table 1 below.

Table 1: Standard Curve Preparation

Standard	GST Concentration (ng/mL)	Assay Buffer (μL)	Volume of 10 ng/mL Standard (μL)	Final Volume (μL)
S7	10	-	1000	1000
S6	8.0	200	800	1000
S5	5.0	500	500	1000
S4	2.5	750	250	1000
S3	1.0	900	100	1000
S2	0.5	950	50	1000
S1	0.25	975	25	1000
S0	0	1000	-	1000

ASSAY PROCEDURE

1. Add 100 μL of Standards and Samples to the corresponding wells on the microplate in duplicate. Incubate at room temperature for one hour. See Scheme 1 below for a suggested plate layout.
2. Dump the contents of the plate and wash each well three times with 300 μL of Wash Buffer. After the final wash, tap the plate on a lint-free paper towel to make sure there is no solution left in the wells.
3. Add 100 μL of the Detection Antibody to each well. Incubate at room temperature for one hour.
4. Wash the plate as in step 2.
5. Add 100 μL of the HRP Conjugate to each well. Incubate at room temperature for 30 minutes.
6. Wash the plate as in step 2.
7. Add 100 μL of TMB Substrate to each well. Allow the color to develop for 30 minutes at room temperature.
8. Stop the reaction by adding 25 μL per well of 3N Sulfuric Acid (H_2SO_4).
9. Read the plate at 450 nm in a microplate reader.

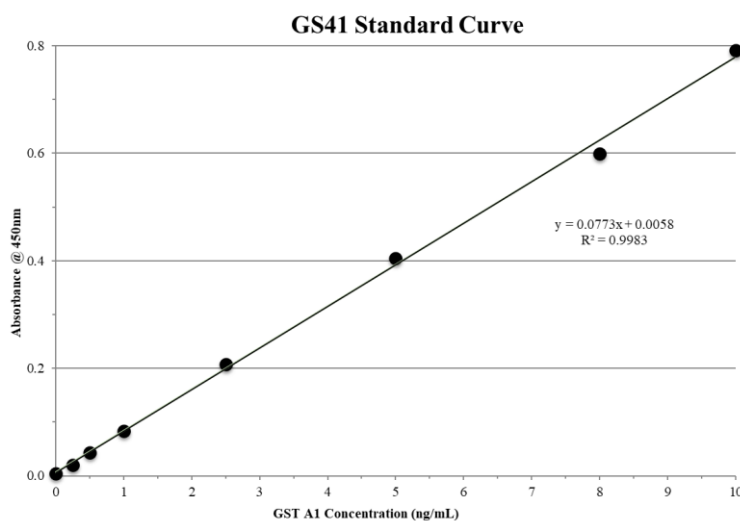
Scheme 1: Suggested Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S ₀	S ₀	U ₁	U ₁	U ₉	U ₉	U ₁₇	U ₁₇	U ₂₅	U ₂₅	U ₃₃	U ₃₃
B	S ₁	S ₁	U ₂	U ₂	U ₁₀	U ₁₀	U ₁₈	U ₁₈	U ₂₆	U ₂₆	U ₃₄	U ₃₄
C	S ₂	S ₂	U ₃	U ₃	U ₁₁	U ₁₁	U ₁₉	U ₁₉	U ₂₇	U ₂₇	U ₃₅	U ₃₅
D	S ₃	S ₃	U ₄	U ₄	U ₁₂	U ₁₂	U ₂₀	U ₂₀	U ₂₈	U ₂₈	U ₃₆	U ₃₆
E	S ₄	S ₄	U ₅	U ₅	U ₁₃	U ₁₃	U ₂₁	U ₂₁	U ₂₉	U ₂₉	U ₃₇	U ₃₇
F	S ₅	S ₅	U ₆	U ₆	U ₁₄	U ₁₄	U ₂₂	U ₂₂	U ₃₀	U ₃₀	U ₃₈	U ₃₈
G	S ₆	S ₆	U ₇	U ₇	U ₁₅	U ₁₅	U ₂₃	U ₂₃	U ₃₁	U ₃₁	U ₃₉	U ₃₉
H	S ₇	S ₇	U ₈	U ₈	U ₁₆	U ₁₆	U ₂₄	U ₂₄	U ₃₂	U ₃₂	U ₄₀	U ₄₀

CALCULATIONS

1. Average all duplicate well absorbance values.
2. Subtract the average absorbance values for the blank wells (S₀) from all other well pairs.
3. Plot a standard curve using the corrected absorbance values of each Standard (y-axis) versus the Standard concentration (x-axis).
4. Determine the concentration of each unknown using the equation of the line.

Figure 1: Typical Standard Curve



REFERENCES

1. Vaubourdolle, M, *et al.*; (1995) *Clinical Chemistry* 41:1716-1719
 2. Awasthi, Y. C, *et al.*; (2004) *Free Radic. Biol. Med.* 37: 607–619
 3. Yang, Y., *et al.*; (2002) *Toxicol. Appl. Pharmacol.* 182: 105–115
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