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 peroxidation[2] 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	15 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. 3 N Sulfuric Acid (H_2SO_4)
4. Deionized Water (dH_2O)

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.

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 1:100 by adding 120 µL of HRP-Conjugate to 12 mL of Assay Buffer.

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)
S_7	10	-	1000	1000
S_6	5.0	500	500	1000
S_5	2.5	750	250	1000
S_4	1.0	900	100	1000
S_3	0.5	950	50	1000
S_2	0.25	975	25	1000
S_1	0.1	990	10	1000
S_0	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 150 µL of TMB Substrate to each well. Allow the color to develop for 30 minutes at room temperature.
8. Stop the reaction by adding 50 µL per well of 3N Sulfuric Acid (H_2SO_4).
9. Read the plate at 450 nm in a microplate reader.

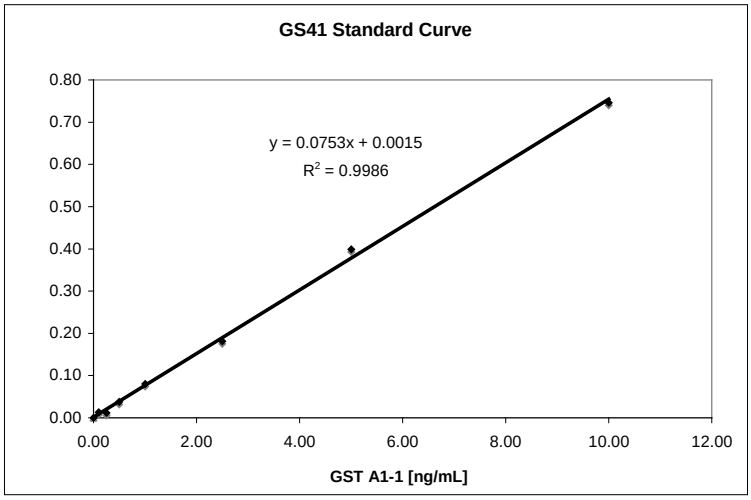
Scheme 1: Suggested Plate Layout

	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 (S_0) 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.

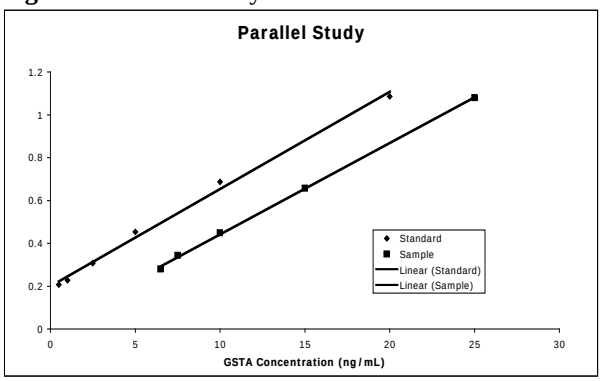
Figure 1: Typical Standard Curve



PARALLEL STUDY

A parallel study is one of the best ways to validate an assay. In this study, a known high sample is linearly diluted and should, when graphed against the standard curve, run parallel to it.

Figure 2: Parallel Study Results



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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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