Microplate Assay for
Glutathione S-Transferase Alpha

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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 2.5 hours to run, plus any required sample preparation time.

MATERIALS PROVIDED

Component	Description	Volume	Storage	Cat no.
Assay Buffer	Buffer used to dilute samples and reagents	100 mL	4°C	GS41a
5x Wash Buffer	Buffer used to wash the plate	40 mL	4°C	GS41b
Standard	2 µg/mL GSTA	30 µL	-20°C	GS41c
Antibody-HRP	Anti-Human-GSTA-HRP	440 µL	-20°C	GS41d
TMB Substrate	Stabilized TMB color reagent	12 mL	4°C	GS41e
Plate	96-well microplate coated and blocked	1 plate	4°C	GS41f

MATERIALS NEEDED BUT NOT PROVIDED

1. Microplate reader with 450 nm filter
2. Polypropylene microcentrifuge tubes
3. Adjustable micropipettes and tips
4. 3 N Sulfuric Acid (H_2SO_4)
5. 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 diluted reagents or standards for future use.

SAMPLE COLLECTION AND 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 are to be diluted in Assay Buffer. Dilution factors of 1:2 and 1:4 are normally sufficient.

REAGENT PREPARATION

1. **Wash Buffer:** Dilute the wash buffer 1:5 with dH_2O. For example, if all the Wash Buffer will be used dilute the 40 mL with 160 mL of dH_2O, giving a total final volume of 200 mL.
2. **Antibody-HRP:** Immediately prior to use, dilute the needed amount 1:25 with Assay Buffer. For example, if all will be used, dilute the complete 440 μL with 10.66 mL of Assay Buffer, giving a total final volume of 11 mL.
3. **Standard:** Perform a 1:100 dilution using Assay Buffer, giving a final concentration of 20 ng/mL. For example, add the 30 μL of Standard to 2.970 mL of Assay Buffer giving a total final volume of 3.0 mL.

STANDARD CURVE PREPARATION

Set up for the standard curve by labeling dilution tubes and dispensing the indicated volumes of Assay Buffer and diluted Standard according to Table 1 below.

Table 1: Standard Curve Preparation
NOTE: *Be sure to dilute the Standard 1:100 as noted in Reagent Preparation first.*

Standard	GST Concentration (ng/mL)	Assay Buffer (µL)	Volume of 20 ng/mL Standard (µL)	Final Volume (µL)
S_6	20	-	1000	1000
S_5	10	500	500	1000
S_4	5.0	750	250	1000
S_3	2.5	875	125	1000
S_2	1.0	950	50	1000
S_1	0.5	975	25	1000
B_0	0.0	1000	-	1000

ASSAY PROCEDURE

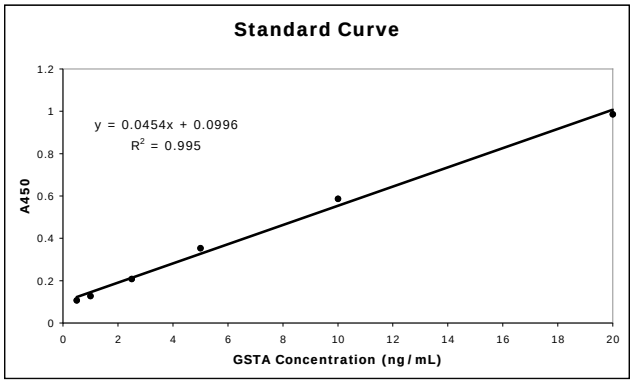
1. Add 100 μL of Standards, Samples, or Blank (B_0) to the corresponding wells on the microplate in duplicate. Incubate at room temperature for one hour.
2. Dump the contents of the plate and wash each well three times with 300 μL of Wash Buffer using a multichannel pipette. After the final wash, tap 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 Antibody-HRP to each well. Incubate at room temperature for one hour.
4. Dump the contents of the plate and wash each well three times with 300 μL of Wash Buffer using a multichannel pipette. After the final wash, tap the plate on a lint-free paper towel to make sure there is no solution left in the wells.
5. Add 100 μL of TMB Substrate to each well. Allow color to develop for 30-40 minutes at room temperature.
6. Stop the reaction by adding 100 μL per well of 3N Sulfuric Acid (H_2SO_4).
7. Read the plate at 450 nm in a microplate reader.

CALCULATIONS

Most plate reader programs are capable of calculating concentrations from a standard curve automatically. However, if this is not possible, follow these steps.

1. Open a spreadsheet program and input the standard concentrations and the average A_{450} of the duplicates in corresponding columns, be sure to subtract the blank A_{450} from each standard.
2. Create a scatterplot graph from the data with the concentration on the x-axis and A_{450} on the y-axis.
3. Add a linear trendline that displays the equation for the line.
4. The equation will be in the form of y = mx + b, where:
    - y is the A_{450}
    - m is the slope of the line
    - x is the concentration
    - b is the y-intercept
5. Subtract the blank A_{450} from each sample, then substitute this value into the equation for y and solve for x, the concentration.
6. If the sample was diluted, be sure to account for this by multiplying the calculated concentration from step 5 by the dilution factor. For example, if a dilution factor of 1:2 was used, multiply the concentration by two for the correct value.

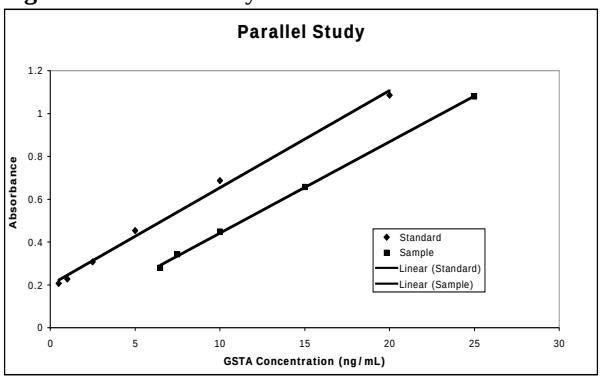
Figure 1: Typical Standard Curve



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., Free Radic. Biol. Med. 37: 607–619, 2004
3. Yang, Y., *et al.* Toxicol. Appl. Pharmacol. 182: 105–115, 2002

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