ELISA Assay for
Glutathione S-Transferase Pi

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INTRODUCTION

The human cytosolic Glutathione S-Transferases (GSTs) are expressed as 18 distinct gene products yet they share a common structural morphology. This immunoassay is specific for GST Pi (GSTP) and exhibits **less than 1% cross reactivity towards the other 17 human cytosolic GST enzymes**. GSTP is considered to be a urinary biomarker for renal cell damage and is localized to the distal convoluted tubules, thin Loop of Henle and the collecting ducts of the kidney[1].

PRINCIPLES OF PROCEDURE

This is a standard sandwich enzyme-linked immunosorbent assay (ELISA). The plate is pre-coated with anti-GSTP 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-GSTP Plate	96-well microplate coated and blocked	1 plate	4°C	GS46a
Assay Buffer	Buffer used to dilute samples and reagents	100 mL	4°C	GS46b
10x Wash Buffer	Buffer used to wash the plate	30 mL	4°C	GS46c
GSTP Standard	100 ng/mL GSTP	320 µL	4°C	GS46d
Detection Antibody	Anti-Human-GSTP	130 µL	4°C	GS46e
HRP-Conjugate	Streptavidin-HRP conjugate	130 µL	4°C	GS46f
TMB Substrate	Stabilized TMB color reagent	20 mL	4°C	GS46g

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 and urine samples, however it can also be used for plasma samples. **NOTE: the use of serum samples for assessing the level of circulating GSTP in blood will give artificially higher levels due to the release of GSTP from platelets during the clotting process[2].**

SAMPLE PREPARATION

It is recommended to do multiple sample dilutions to ensure that the concentration falls within the accepted range for the assay. Urine samples should be diluted at least 1:2 in Assay Buffer for best results. Serum and Plasma samples should not be run neat and are recommended to be run diluted at least 1:3 in Assay Buffer.

REAGENT PREPARATION

1. **GSTP Standard:** Immediately prior to use, dilute 1:10 by adding 300 μL of Standard to 3 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	GSTP Concentration (ng/mL)	Assay Buffer (µL)	Volume of 10 ng/mL Standard (µL)	Final Volume (µL)
S_7	10	-	1000	1000
S_6	8.0	200	800	1000
S_5	5	500	500	1000
S_4	2.5	750	250	1000
S_3	1	900	100	1000
S_2	0.5	950	50	1000
S_1	0.25	975	25	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 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.

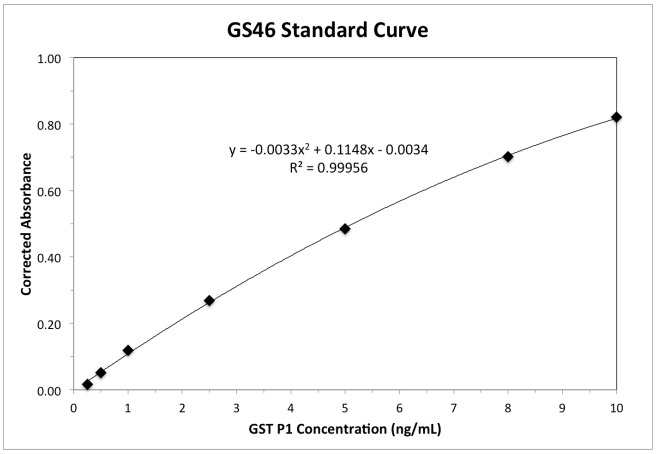
Scheme 1: Suggested Plate Layout (S=Standards; U=Unknown [Samples])

	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. Calculate the average absorbance value for all duplicate wells.
2. Subtract the average absorbance value for the blank wells (S_0) from all other duplicate well pairs.
3. Plot the corrected absorbance values (y-axis) versus concentration (x-axis) for each Standard and generate a Standard Curve using a 3-Parameter Polynomial Regression model ($y=ax^2+bx+c$) (Figure 1). This model has been shown to provide a more precise and less biased fit for ELISAs[3].
4. Determine the concentration of the unknowns (Samples) using the formula: $$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

Figure 1: Typical Standard Curve



SENSITIVITY

The Limit of Detection (LOD) of this assay was calculated to be 0.28 ng/mL, which was determined by adding three standard deviations to the mean optical density value of 24 blank sample replicates then calculating the corresponding concentration from the standard curve. By using this LOD, a concentration as low as 0.56 ng/mL can be determined in a urine sample diluted 1:2 in assay buffer.

PARALLEL STUDY

The validation process of any immunoassay examines the potential existence of interfering or cross-reacting substances in a sample matrix. To test for this, an immunoassay is examined for its ability to demonstrate parallelism. This immunoassay was validated by determining parallelism in both urine and serum samples. A spiked urine sample (Figure 2) and unspiked serum sample (Figure 3) were serially diluted and plotted against a serially diluted 10 ng/mL GSTP Standard. The resulting lines should be close to parallel if no interfering or cross-reacting substances are present in the sample matrix.

Figure 2: Parallel Study Results-Urine

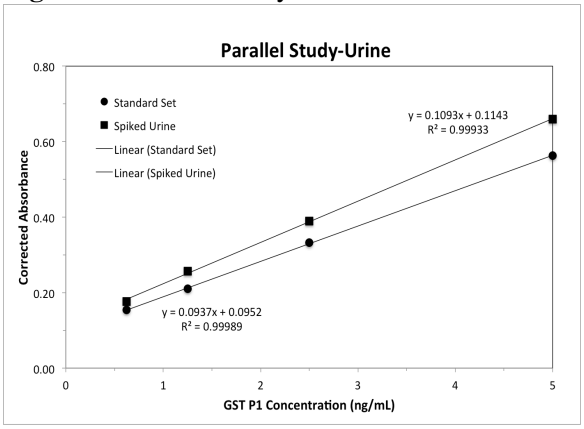
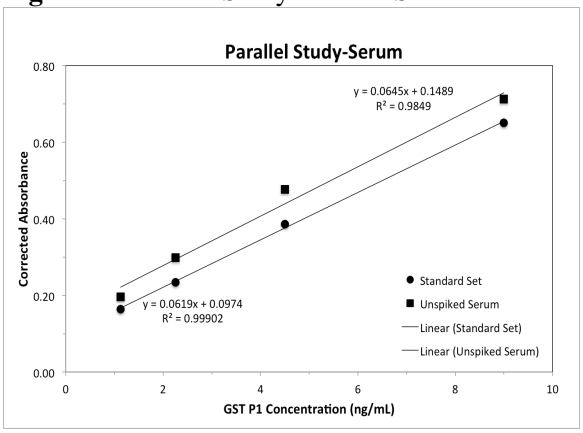


Figure 3: Parallel Study Results-Serum



SPIKE RECOVERY

A spike recovery experiment was performed with a serum sample that was spiked with three concentrations of GSTP: 10 ng/mL, 50 ng/mL and 100 ng/mL (Table 2). The percent spike recovery was calculated by correcting for the endogenous GSTP in the sample then using the following equation: (measured GSTP/expected GSTP) × 100. Percent Spike Recovery should fall between 90-110%. Comparable results were obtained when a spike recovery experiment was performed with urine.

Table 2: Spike Recovery Results-Serum

Sample	Spike	Expected (ng/mL)	Measured (ng/mL)	Percent Recovery
Serum	Low	10	9.86	98.6 %
	Mid	50	45.48	91.0 %
	High	100	105.0	105.0 %

REFERENCES

1. Sundberg, A. G. M, *et al*.; (1994) Nephron, 66:162-169
2. Mulder, T.P., *et al*.; (1997) Cancer. 80(5): 873-880
3. Herman, R.A., *et al*.; (2008) Journal of Immunological Methods, 339: 245-258

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