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NWLSS™ Gluathione-S-Transferase Alpha GST-α ELISA

Product NWK-GSTAHR1
For Research Use Only

ELISA assay system for measurement of Glutathione-S-Transferase Alpha (GST-α) in human and rat samples.

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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. It also conjugates GSH to 4-hydroxynonenal, a product of lipid peroxidation and is an important player in cellular antioxidant defense mechanisms.

Intended Use:
This kit is intended for the quantification of human Glutathione S Transferase Alpha (GSTA) in biological samples where GSTA may be present.

Test Principle:
This assay utilizes a sandwich immunoassay (ELISA) strategy. A plate is provided pre-coated with anti GSTA. Samples and standards are added to the plate wherein GSTA is captured. After washing, an anti GSTA-HRP conjugate is added for detection. After another washing TMB substrate addition results in a blue color development that is directly proportional to the amount of GSTA in the original samples or standards. Addition of an acid stop solution causes a color change to yellow where absorbance is read at 450 nM.

General Specifications:

Format:	96 well Sandwich ELISA
Number of tests:	Triplicate = 24 Duplicate= 40
Specificity:	Human Glutathione-S-Transferase
Sensitivity:	.325 ng/mL
Effective Range:	0.325 ng/mL - 20 ng/mL

Kit Contents

Microwells precoated with Anti Human GSTA	1 X 96 wells
Human GSTA Standard (2ug/mL)	1 X 30 µL
5X Wash Buffer:	1 X 40 mL
TMB Substrate:	1 X 12 mL
Anti GSTA-HRP Conjugate:	1 X 440 µL
Assay Buffer:	1 X 100 mL

Required Materials Not Provided:

Adjustable pipettes with disposable tips.

Deionized water.

Microcentrifuge tubes.

3M sulfuric acid.

Required Instrumentation:

Microtiter plate reader with 450 nm capability.

Warnings, Limitations, Precautions:

Individual components may be harmful if swallowed, inhaled or absorbed through the skin. Contact should be minimized through the use of gloves and standard good laboratory practices. If contact with skin or eyes occurs, rinse the site immediately with water and consult a physician.

Storage Instructions:

Store all components except Standard and HRP Conjugate at 4°C until immediately before use.

Store Standard and HRP Conjugate at –20°C until immediately before use.

Diluted reagents and standards are not suitable for storage and re-use.

Assay Preparation

1. Determine the number of wells required to assay standards, samples and controls for the appropriate replicate value and create an assay template showing positioning of standards, controls and samples. Include blank wells also.

2. Bring all samples and reagents to room temperature before use. To avoid condensation, do not open foil pouches containing the microtiter strips until after they have reached room temperature.

4. Next remove the required number of strips and place in the frame supplied. Return unused wells to the storage bag, seal and store at 2-8ºC.

Reagent Preparation:
The following instructions are based on the user using the entire kit at one time. If portions of the kit are to be used at a later time, smaller quantities may be prepared saving the remaining stock for later use.

Assay Buffer is supplied ready to use.

TMB Substrate is supplied ready to use.

5X Wash Buffer
Add the contents of the 5X Wash Buffer to 160 mL deionized H_2O, mix well and label as **Working Wash Buffer.**

Anti GSTA-HRP Conjugate
Determine the amount of Anti GSTA-HRP Conjugate required then dilute 1:25 with Assay Buffer. For the entire plate dilute 440 uL Anti GSTA-HRP Conjugate with 10.66 mL Assay Buffer. Label as **Diluted Anti GSTA-HRP Conjugate.**

Standard Preparation:
Standard Supplied: Anti Human GSTA: 1 x 30 µL at 2 µg/mL

Standard 7 (S_7): Create a 20 ng/mL Standard by diluting 2 ug/mL stock standard 1:100 using Assay Buffer. If entire plate will not be tested at once dilute only the requisite volume for one standard curve. Example: for triplicate standards add 8 uL stock standard to 792 uL Assay Buffer. Label as 20 ng/mL GSTA Standard.

Standard 6 (S_6): Add 400 µL of S_7 to 400 µL Assay Buffer and *vortex. The* S_5 *concentration is now* ***10 ng/mL.***

Standard 5 (S_5): Add 400 µL of S_6 to 400 µL Assay Buffer and *vortex. The* S_4 *concentration is now* ***5 ng/mL.***

Standard 4 (S_4): Add 400 µL of S_5 to 400 µL Assay Buffer and vortex. *The* S_3 *concentration is now* ***2.5 ng/mL.***

Standard 3 (S_3): Add 400 µL of S_4 to 400 µL Assay Buffer and vortex. *The* S_2 *concentration is now* ***1.25 ng/mL.***

Standard 2 (S_2): Add 400 µL of S_3 to 400 µL Assay Buffer and vortex. *The* S_1 *concentration is now* ***0.625 ng/mL.***

Standard 1 (S_1): Add 400 µL of S_2 to 400 uL Assay Buffer and Vortex. *The* S_1 *concentration is now* ***0.313 ng/mL.***

Sample Handling/Preparation

This kit is designed for use in testing GSTA in serum, plasma and urine samples. If necessary, samples can be diluted in Assay Buffer. However, since the amount of GST will vary among sample types, it is recommended that the user determine the optimal dilution for their specific project. Diluting samples 1:2 to 1:4 with Assay Buffer is usually a good starting point.

Assay Protocol:

1. Add 100 µL of **Standard, Samples or Blank** to each well.

2. Invert plate and empty contents. Pat dry upside-down on a lint free towel. Wash plate with 300µL Working Wash Buffer 3 times allowing plate to stand 2 minutes per wash.

3. Add 100 µL of **Diluted Anti GSTA-HRP Conjugate** to each well except blank. Add 100 µL Assay Buffer to blank wells. Allow plate to stand for 1 hour (60 min) at Room Temperature.

4. Invert plate and empty contents. Pat dry upside-down on a lint free towel. Wash plate with 300µL Working Wash Buffer 3 times allowing plate to stand 2 minutes per wash.

5. Add 100 µL TMB Substrate to each well, allow to stand for 30-40 minutes allowing blue color to develop maximally in high standard wells.

6. Add 100 µL of 3M sulfuric acid to each well to stop the reaction. This changes the color to yellow.

7. Record the absorbance at 450 nm using a plate reader.

Data Analysis

1. Average the reagent blank absorbance values and subtract this average from each well. Most modern microplate readers are capable of doing this automatically.

2. Average standard replicates (S_1 through S_7) and plot Absorbance at 450 nm VS Concentration GSTA to obtain the standard curve.

3. The standard curve obtained should be linear such that samples can be analyzed according to the normal line equation:

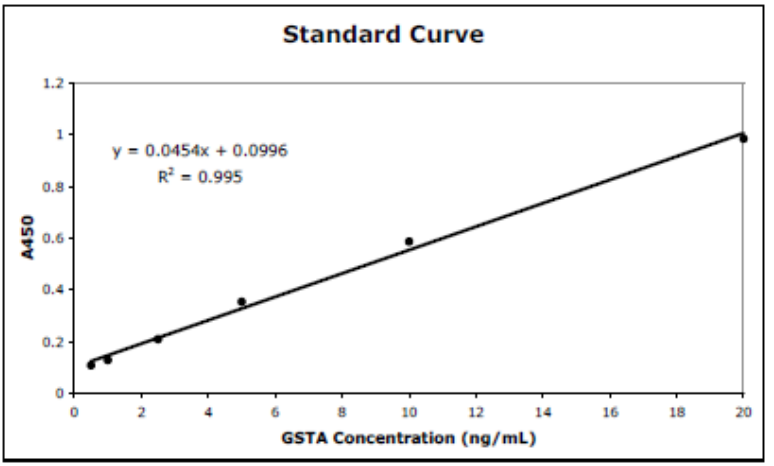
$y = mx + b$

else

$[GSTA] = (y - b)/m$

Figure 1 shows a typical standard curve obtained when plotting ABS450 VS GSTA Concentration in this fashion.

Figure 1.



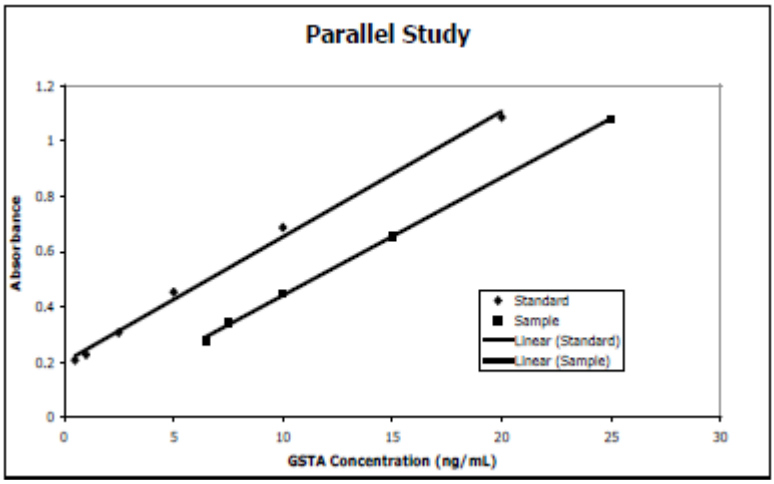
4. Remember to account for any dilution factor incurred by samples prior to assay.

Specificity & Crossreactivity:

Specificity Validation:

A sample with some quantity GSTA was diluted and compared against known values of human GSTA. When compared in this fashion the diluted sample plot should be parallel to the standard curve plot. See Figure 2.

Figure 2.



Cross reactivity:

In addition to being specific for Human GSTA, this assay has been shown to cross-react with Rat GSTA.

REFERENCES

1. Awasthi, Y.C. et. Al., Regulatoin of 4-hydroxynonenal-mediated signaling by glutathione-S-transferases. *Free Radical Biology and Medicine* 37:607 -619, 2004.

2. Yang, Y. et. Al., Role of alpha Class Glutathione S-Transferases as Anti-oxidant Enzymes in Rodent Tissues *Toxicology and Applied Pharmacology* 182:105-115, 2002.

3. Vaubourdolle, M et. al., Plasma α-glutathione S-transferase assessed as a marker in patients with chronic hepatitis C. *Clinical Chemistry* 41:1716-1719, 1995.

User Notes:

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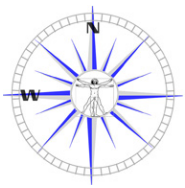

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