Fluoro GST

Fluorescence Glutathione S-Transferase Activity Kit

GSTR100-2

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Introduction

The Glutathione S-Transferase (GST) family of isozymes function to detoxify and neutralize a wide variety of electrophilic molecules by mediating their conjugation with reduced glutathione[1]. Human GSTs are encoded by 5 gene families, expressing in almost all tissues as four cytosolic and one microsomal forms. Dividing the family by isoelectric points, the basic alpha (pI 8–11), the neutral mu (pI 5–7) and acidic pi (pH<5) classes are populated by additional subclasses, each isozyme displaying differential specificity for given electrophilic molecules[2].

Given its pivotal role in ameliorating oxidative stress/damage, GST activity has been repeatedly investigated as a biomarker for arthritis, asthma, COPD, and multiple forms of cancer, as well as an environmental marker[3-7]. Examination of GST isoforms and activity in human cancers, tumors and tumor cell lines has revealed the predominance of the acidic pi class. Furthermore, this activity is thought to substantially contribute to the innate or acquired resistance of specific neoplasms to anticancer therapy[8,9].

Assay Principle

The Fluoro GST kit is designed to quantitatively measure the activity of GST present in a variety of samples. Please read the complete kit insert before performing this assay. A GST standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. The kit utilizes a non-fluorescent molecule that is a substrate for the GST enzyme that covalently attaches to glutathione (GSH) to yield a highly fluorescent product. Mixing the sample or standard with the supplied Detection Reagent and GSH and incubating at room temperature for 30 minutes yields a fluorescent product that is read at 460 nm in a fluorescent plate reader with excitation at 390 nm. The activity of the GST in the sample is calculated, after making a suitable correction for any dilution of the sample, using software available with most fluorescence plate readers.

We have provided a 96 well plate for measurement but this assay is adaptable for higher density plate formats. The end user should ensure that their HTS black plate is suitable for use with these reagents prior to running samples.

Applications

The Fluoro GST kit is designed to detect Glutathione S-Transferase activity in biological samples such as serum, plasma, urine and cell lysates.

Schematic Representation of Glutathione S-Transferase Fluorescence Detection Kit

- Prepare standards in the diluted assay Buffer.
- Prepare biological samples as appropriate.
- **Set up the following control**:

  Pipette 50 µL of Assay Buffer into duplicate wells designated as the zero standard

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Pipette 50 µL of samples and standards into all wells according to plate layout.

⇩

Add 25 µL of the Detection reagent to all wells

⇩

Add 25µL of GSH to all wells

⇩

Incubate reaction for 30 minutes at RT

⇩

Read fluorescence at excitation 370-410nm and emission at 460nm

Storage

Store components at 2-8 °C upon arrival.

Warnings and Precautions

1. For Research use only. Not for use in diagnostic procedures.
2. Practice safe laboratory procedures by wearing gloves, protective clothing and eyewear.
3. Dimethyl sulfoxide is a powerful aprotic organic solvent that has been shown to enhance the rate of skin absorption of skin-permeable substances. Wear protective gloves when using the solvent especially when it contains dissolved chemicals. NOTE: DMSO can dissolve certain plastics used in troughs used for holding solutions for multichannel pipettes,
4. As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

Catalog #GSTR100-2 Contents and Storage

1. Black Half Area Well Plate (Part# 90036**):** Store at 2-8°C upon arrival.

Well volume (µL)	Well Depth (mm)	Plate Length (mm)	Plate Width (mm)	A1 Row Offset (mm)	A1 Column Offset (mm)
190	10.54	127.8	85.5	11.2	14.3

2. **Glutathione S-Transferase Standard (Part# 90041):** 1 vial (50 µL) Equine Glutathione S-Transferase at 10 mU/mL in a special stabilizing solution. Store at 2-8°C upon arrival.
3. GST Detection Reagent (Part# 90037): 1 vial GST detection substrate stored in a desiccator. Store at 2-8°C upon arrival.

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4. Dry DMSO (Part# 90038): 1 bottle (2 mL) Dry Dimethyl sulfoxide solvent over molecular sieves. DMSO, when stored at 4°C, will freeze. Can be stored tightly capped at room temperature.

5. **Assay Buffer (Part# 90039):** 1 bottle (45 mL) Phosphate buffer containing proteins and stabilizers. Store at 2-8°C upon arrival.

6. **Glutathione (GSH) (Part# 90040):** 1 vial (300 µL) Glutathione supplied as a 20 mM stable solution. Store at 2-8°C upon arrival.

Activity Standardization
The Glutathione S-Transferase standard used in this kit has been calibrated using an enzymatic method adapted from reference 4.

Materials required but not supplied

1. Deionized or distilled water.

2. Fluorescence 96 well plate reader capable of reading fluorescent emission at 460 nm, with excitation at 390 nm. Please contact your plate reader manufacturer for suitable filter sets.

   The sensitivity of fluorescent assays is dependent on the capabilities of the plate reader. If your plate reader has adjustable gain you can modify the signals obtained from the assay by increasing or decreasing the gain settings, by changing the aperture settings for monochromator based readers, or by changing the band pass width of the emission and/or excitation filters on some readers. Please review the plate reader manual for details.

   Signals expressed by plate readers are Relative Fluorescent Units (RFU) and the values given in the protocol were obtained on our plate readers. The RFU numbers you obtain may be different from these, but the assay results should be similar.

   Software for converting raw relative fluorescent unit (FLU) readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

Sample Types and Preparation

This assay has been validated for human urine, serum, EDTA, heparin plasma, toadfish liver (*Opsanus tau*) and oyster hemolymph samples. Most cell lysates should also be compatible. Samples that are not clear or that contain visible particulate should be centrifuged prior to using.

GST activity varies across tissues and species, however we expect this kit to measure GST activity from sources other than human. The end-user should evaluate recoveries of GST activity in samples from other species being tested.

Any samples requiring larger dilutions or with GST activities outside the standard curve range should be diluted further with Assay Buffer to obtain readings within the standard curve.

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Urine Samples

Samples that are not clear or that contain visible particulate should be centrifuged prior to using. Urine samples should be diluted ≥ 1:2 in Assay Buffer by adding one part of urine to one part of Assay Buffer. Sample values should be normalized for urinary volume to urinary creatinine levels.

Serum and Plasma Samples

Samples that are not clear or that contain visible particulate should be centrifuged prior to using. Fresh serum or EDTA and heparin plasma are separated by centrifugation at 600 x g for 10 minutes. Transfer the serum or plasma from the red blood cells into fresh tubes. The serum or plasma may be stored at -80°C or analyzed immediately. Serum or plasma should be diluted with Assay Buffer at a dilution of ≥1:2.

Cell Lysates

Washed cell pellets are resuspended at 10-40x10^6 cells/mL in Assay Buffer (we used Jurkats at 10 x 10^6 cells/mL) and are lysed by vigorous vortexing, freeze/thaw cycling or other suitable disruption method. Resulting centrifuged lysate supernatants are measured at appropriate dilutions. The protocol might require adjustment for other cell types. If protein determinations are to be made on the samples, we would recommend using higher number of cells and lysing in your normal PBS-based lysis buffer and determining protein concentration, prior to additional dilutions in Assay Buffer to measure GST activity.

Use all samples within 2 hours of dilution.

Assay Protocol

A. Reagent Preparation

Standard Preparation. GST Standards are prepared by labeling seven test tubes as #1 through #7. Briefly spin vial of standard in a micro centrifuge to ensure contents are at bottom of vial. Pipette 380 µL of Assay Buffer into tube #1 and 200 µL into tubes #2 to #7. Carefully add 20 µL of the Glutathione S-Transferase Standard to tube #1 and vortex completely. Take 200 µL of the GST solution in tube #1 and add it to tube #2 and vortex completely. Repeat these serial dilutions for tubes #3 through #7. The concentration of GST in tubes 1 through 7 will be 500, 250, 125, 62.5, 31.25, 15.61 and 7.81 mU/mL.

Use all Standards within 1 hour of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Buffer Volume (µL)	**380**	200	200	200	200	200	200
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Volume of Addition (µL)	**20**	200	200	200	200	200	200
Final Conc (mU/mL)	500	250	125	62.5	31.25	15.625	7.81
Dilution	1:20	1:2	1:2	1:2	1:2	1:2	1;2

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Detection Reagent. Remove the vial of Detection Reagent from the desiccator and add 300 µL of the dry DMSO to the vial. Vortex thoroughly. Store any unused reconstituted Detection Reagent at 4°C in the desiccator for no longer than 2 weeks.

Dilute one part of reconstituted Detection Reagent 1:10 into nine parts of Assay Buffer. 150 µL of Detection Reagent should be diluted with 1.35 mL of Assay Buffer to use half the plate. Discard any excess diluted Detection Reagent.

Glutathione. Dilute one part Glutathione stock provided 1:10 into nine parts of Assay Buffer. 150 µL of Glutathione stock should be diluted with 1.35 mL of Assay Buffer to have enough GSH to be able to read half the plate. Discard any excess diluted glutathione.

B. Assay Protocol

1. Use the plate layout sheet at the end of the protocol to aid in proper sample and standard identification.
2. Pipette 50 µL of treated samples or standards into duplicate wells in the plate.
3. **Control**: Pipette 50 µL of Assay Buffer into duplicate wells as the Zero standard.
4. Add 25 µL of the Detection Reagent to each well using a repeater or multichannel pipette.
5. Add 25 µL of GSH to each well using a repeater or multichannel pipette.
6. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
7. Incubate at room temperature for 30 minutes.
8. Read the fluorescent signal from each well in a plate reader capable of reading the fluorescent emission at 460 nm with excitation at 370-410 nm. Please contact your plate reader manufacturer for suitable filter sets.
9. Use the plate reader's built-in 4PLC software capabilities to calculate GST activities for each sample..

Calculation of Results

Average the duplicate FLU readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean FLUs for the zero standard. The concentrations obtained should be multiplied by the dilution factor to obtain neat sample values.

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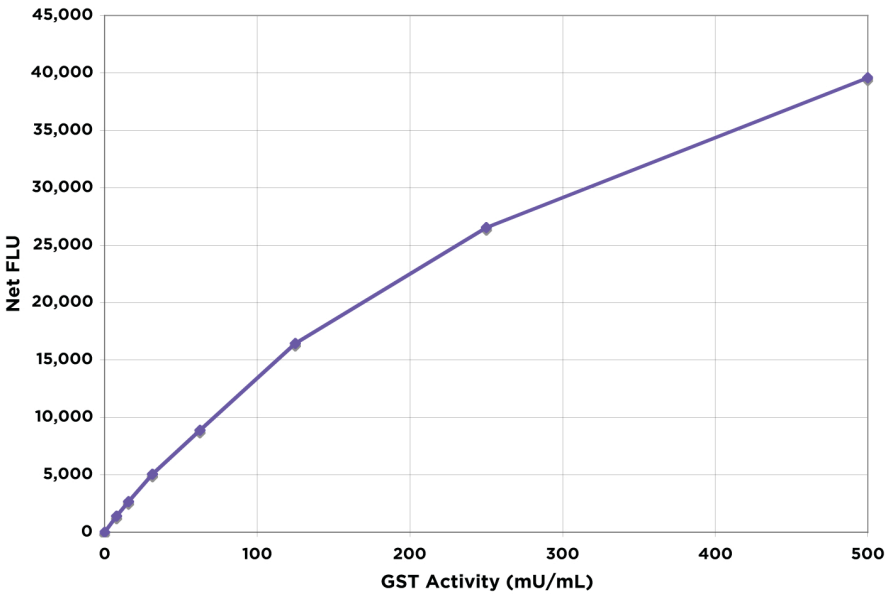
Typical Data

Sample	Mean FLU	Net FLU	GST Activity mU/mL
Zero	5,860	0	0
Standard 1	45,765	39,905	500
Standard 2	32,561	26,01	250
Standard 3	22,093	16,33	125
Standard 4	14,986	9,126	62.5
Standard 5	10,959	5,099	31.25
Standard 6	8,674	2,814	15.61
Standard 7	7,471	1,611	7.81
Sample 1	31,089	25,229	229.5
Sample 2	9,497	3,637	21.26

Always run your own standard curve for calculation of results.
Do not use this data.

Typical Standard Curve



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Validation Data

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the FLUs for twenty wells run for each of the zero and standard #7. The detection limit was determined at two (2) standard deviations from the zero along the standard curve. **Sensitivity was determined as 2.70 mU/mL.**

The Limit of Detection was determined in a similar manner by comparing the FLUs for twenty wells run for each of the zero and a low concentration serum sample. **The Limit of Detection was determined as 1.90 mU/mL.**

Linearity

Linearity was determined by taking Jurkat cell lysates at 10 x 106 and 0.8 x 106 cells/mL and mixing in the ratios given below. The measured activities were compared to the expected values based on the ratios used.

Low Cell #	High Cell #	Expected Activity (mU/mL)	Observed Activity (mU/mL)	% Recovery
100%	0%	40.4	--	--
80%	20%	123.3	122.4	99.3
60%	40%	206.2	217.2	105.3
40%	60%	289.1	297.	102.7
20%	80%	372.1	395.1	106.2
0%	100%	455.0	--	--
			Mean Recovery	103.4%

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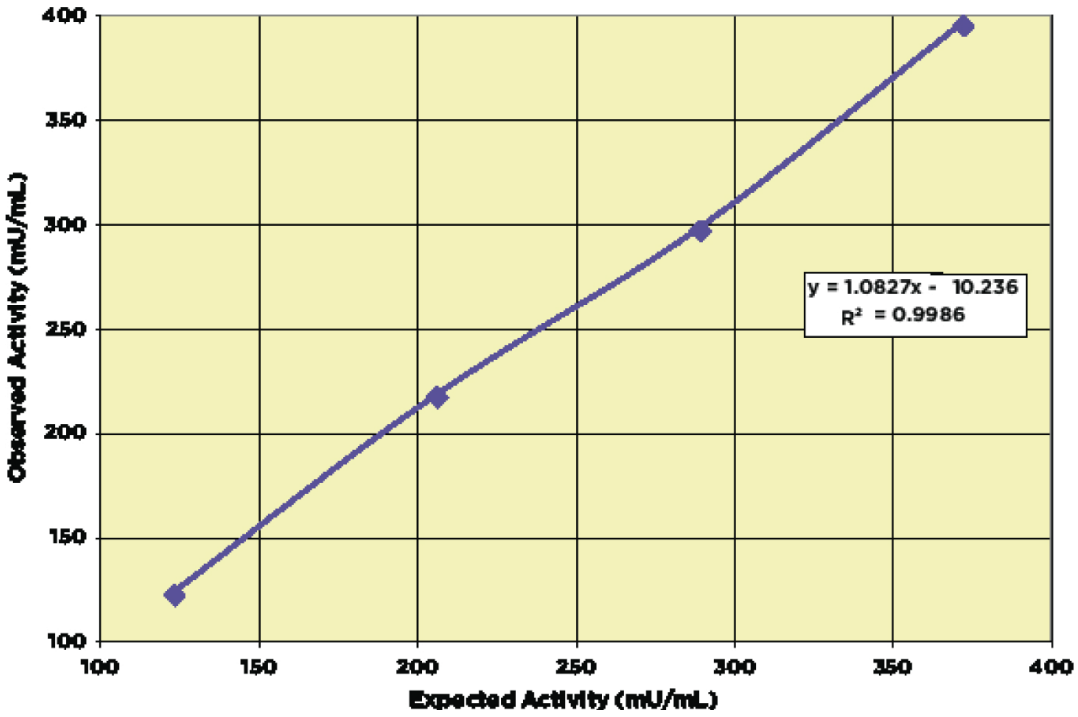

Intra Assay Precision

Four serum samples were diluted 1:2 with Assay Buffer and run in replicates of 16 in an assay. The mean and precision of the calculated GST activities were:

Sample	GST Activity (mU/mL)	%CV
1	315.9	4.6
2	221.2	5.6
3	88.2	4.2
4	22.7	6.6

Inter Assay Precision

Four serum samples were diluted 1:2 with Assay Buffer and run in duplicates in twenty assays over multiple days by four operators. The mean and precision of the calculated GST activities were:

Sample	GST Activity (mU/mL)	%CV
1	291.7	12.6
2	218.5	11.0
3	89.6	10.4
4	23.0	15.9

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Interferences

A variety of solvents and detergents were tested as possible interfering substances in the assay. Approximately 10% change was seen in the GST activity in the presence of 1% methanol or DMSO in the sample. Three detergents were also tested, Triton X-100, Tween 20 and SDS. At 0.01% concentration in the sample both SDS and Tween showed no change in activity, whereas Triton showed >47% decrease at 0.01%.

Bilirubin levels of 2.5 µg/mL in the sample showed < 5% decrease in GST activity.

End-Point Versus Kinetic Activity

The assay can also be run as a kinetic assay. A human serum sample was read in both an end point and in a kinetic assay. In the end point measurement it had a reading of 12.12 mU/mL and in the kinetic assay a reading of 11.92 mU/mL. A typical standard curve for the kinetic assay is shown below:

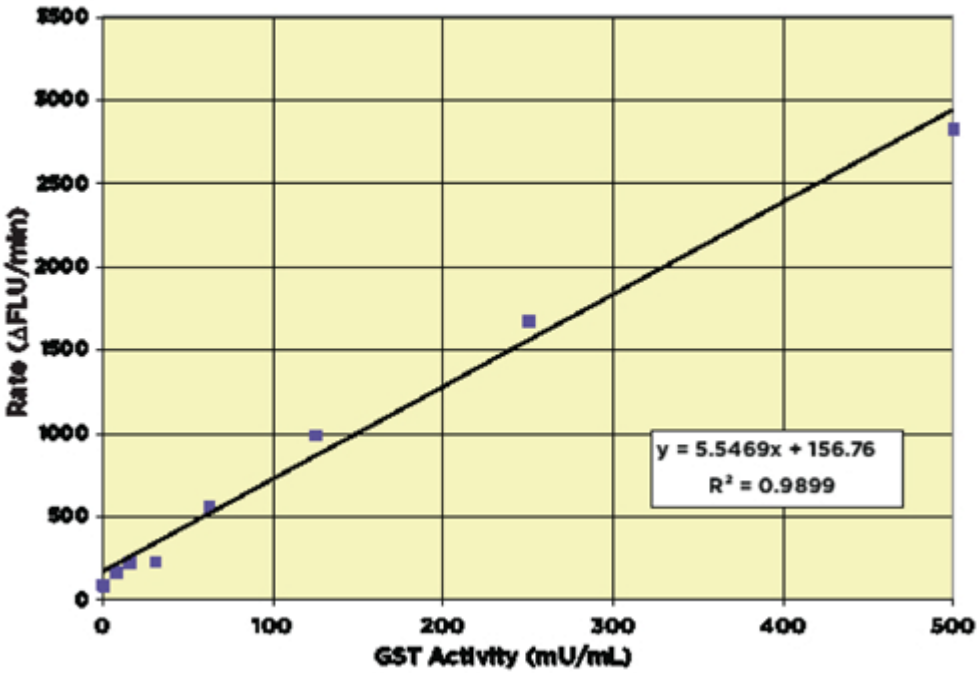

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Plate Layout Sheet:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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