DetectX™

Glutathione S-Transferase Fluorescent Activity Kit

Catalog Number K008-F1

LUMINOS®
INTERACTIVE ASSAY SOLUTIONS

FEATURES

- Super sensitive assay for GST activity
- Fluorescent assay, perfect for HTS
- Simple end point assay with kinetic option

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. 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.

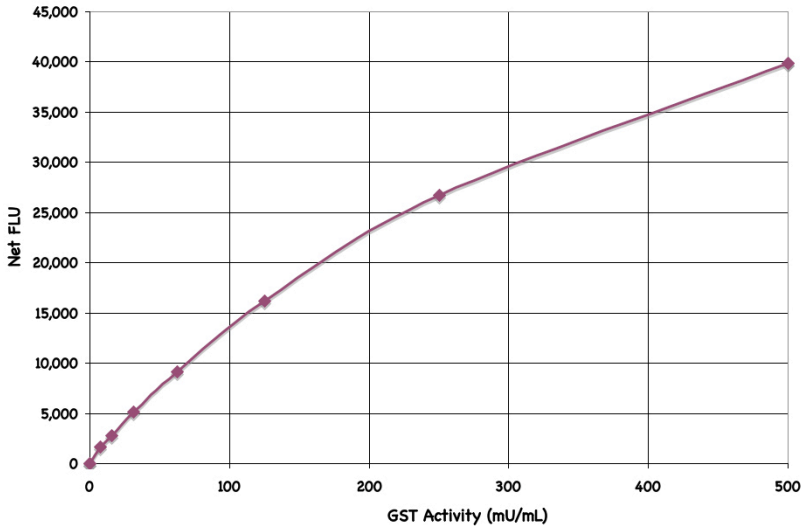
Given its pivotal role in ameliorating oxidative stress/ damage, GST activity has been repeatedly investigated as a biomarker for arthritis, asthma, COPD, and multiple forms of cancer, as well as an environmental marker. Examination of GST isoforms and activity in human cancers, tumors and tumor cell 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.

The Luminos® DetectX™ Glutathione S-Transferase (GST) Activity kit is designed to quantitatively measure GST activity in a variety of samples. A GST standard is provided to generate a standard curve for the assay. The kit utilizes non-fluorescent molecule that covalently binds to the free thiol group on GSH, a product of the GST-catalyzed reaction, to yield a highly fluorescent product. After mixing the sample or standard with GSH, the fluorescent substrate and incubating at room temperature, the fluorescent product is read at 460 nm in a fluorescent plate reader with excitation at 390 nm.

SAMPLE TYPES

Serum, plasma, and cell lysate samples have been validated with this assay.

TYPICAL DATA



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SAMPLE PREPARATION

Detailed protocols for all samples are included in the kit insert.

ASSAY PROTOCOL

- Pipet 50 µL of samples or standards into duplicate wells in the microtiter plate.
- Add 25 µL of GSH and 25 µL of Detection Reagent to each well. Incubate at room temperature for 30 minutes.
- Read GST activity in a fluorescent plate reader at 460 nm (Exc=390nm).

SENSITIVITY

Sensitivity was determined as 2.70 mU/mL GST activity. (Standardized against colorimetric CDNB reaction)

LINEARITY

Linearity was determined by taking 2 Jurkat cell lysate samples, one with a high number of cells/mL and one with a lower level of cells/mL, and mixing them in the ratios given below:

Low Cell # %	High Cell # %	Expected Activity	Observed Activity	% Recovery
80%	20%	123.3	122.4	99.3%
60%	40%	206.2	217.2	105.3%
40%	60%	289.1	297.1	102.7%
20%	80%	372.1	395.1	106.2%
			Mean Recovery	**102.3%**

INTRA ASSAY PRECISION

Four native samples were run in replicates of n=16. The mean and standard deviation of the calculated GST activities were:

Sample	GST Activity (mU/mL)	Std. Dev. (mU/mL)	Total %CV
1	315.9	14.6	4.6
2	221.2	12.3	5.6
3	88.2	3.7	4.2
4	22.7	1.5	6.6

INTER ASSAY PRECISION

Five native samples were run in duplicates in twenty assays over multiple days by four operators. The mean and standard deviation of the calculated GST activities were:

Sample	GST Activity (mU/mL)	Std. Dev. (mU/mL)	Total %CV
1	291.7	36.8	12.6
2	218.5	24.1	11.0
3	89.6	9.3	10.4
4	23.0	3.7	15.9

SAMPLE VALUES AND END POINT VS KINETIC

20 random human serum, heparin and EDTA plasma samples were tested in the assay. Values ranged from 26.8 to 59.8 mU/mL with a average of 39.1 mU/mL. 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.

For details concerning this kit or to order any of our products please contact us:
734-677-1774 • info@LuminosAssays.com • www.LuminosAssays.com