Glutathione S-Transferase Fluorescent Activity Kit

Catalog Number EIAGSTF (96 tests)

Rev 1.0

For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Glutathione S-Transferase Fluorescent Activity Kit is a fluorescent activity assay designed to measure glutathione S-transferase (GST) activity in a variety of samples. The kit uses a proprietary non-fluorescent molecule that is a GST substrate which covalently binds to glutathione (GSH) to produce a fluorescent product (390 nm excitation, 460 nm emission). The assay can be run as an end point assay, or as a kinetic activity assay.

This assay measures the activity of GST in serum, plasma (EDTA and heparin), urine, and cell lysates. The assay was validated with human GST, but is expected to measure GST activity in samples from other species. The assay has been tested successfully with toadfish liver (*Opsanus tau*) and oyster hemolymph samples.

The 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 expressed in almost all tissues as four cytosolic and one microsomal form.

Contents and storage

Kit and components are shipped at –20°C. Upon receipt, store the kit at –20°C. Once open, store the kit at 4°C and use within 2 weeks.

Components	Quantity
Glutathione S-Transferase Standard; 10 U/mL equine glutathione S-transferase in a special stabilizing solution	50 µL
Assay Buffer	45 mL
Black 96-well Half Area Plate	1 plate
GST Detection Reagent; reconstitute with Dry DMSO	1 vial
Dry DMSO (dimethyl sulfoxide)	2 mL
Glutathione (GSH); supplied as a 20 mM stable solution	300 µL

Materials required but not supplied

- Microtiter plate reader with software capable of measurement at or near 460 nm, with excitation at 390 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution

Procedural guidelines

Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Sample preparation guidelines

- Avoid the use of hemolyzed or lipemic sera.
- Collect samples in pyrogen/endotoxin-free tubes.
- Separate fresh serum or EDTA and heparin plasma samples by centrifugation at 600 × *g* for 10 minutes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Prepare cell lysates

1. Wash cell pellets and resuspend in Assay Buffer to 10–40 × 10^6 cells/mL.
2. Lyse cells by vigorous vortexing, freeze-thaw cycling or other suitable disruption method.
3. Centrifuge samples and collect the supernatant for analysis.

Note: If protein determinations are to be made on the samples, use a greater number of cells. Lyse the cells in a PBS-based lysis buffer and determine protein concentration prior to dilution in Assay Buffer.

Dilute samples

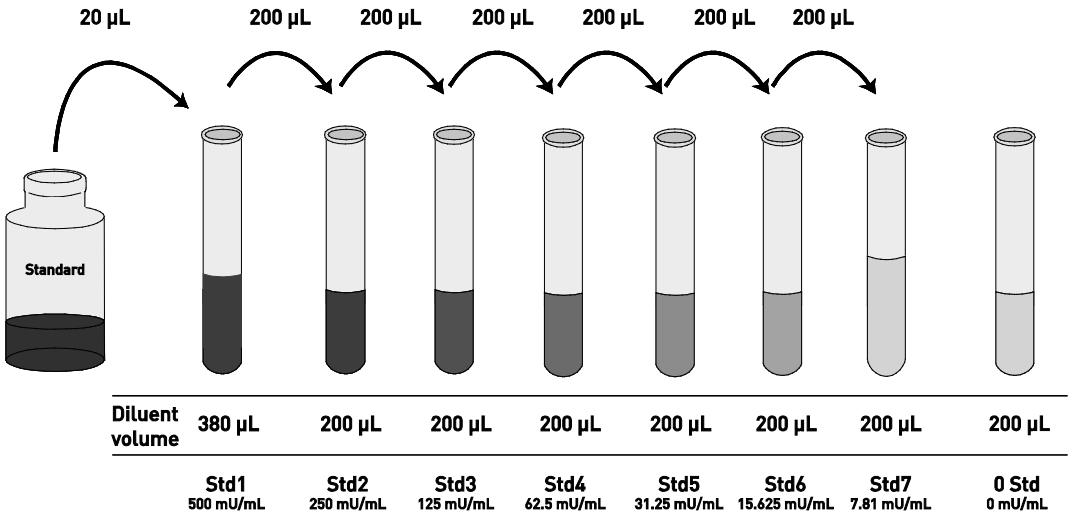
Sample activity should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

- Dilute **serum and plasma** samples ≥1:2 in Assay Buffer.
- Dilute **urine** samples ≥1:2 in Assay Buffer.
  Note: A Creatinine Urinary Detection Kit (Cat. No. EIACUN) is available for measuring urine creatinine for normalization of GST levels to urine creatinine levels.
- Perform dilution of **cell lysate** in Assay Buffer.
- Use all samples within **2 hours** of dilution.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Briefly centrifuge the vial of standard to ensure the contents are at the bottom of vial.
2. Add 20 µL GST Standard to one tube containing 380 µL Assay Buffer and label as 500 mU/mL GST.
3. Add 200 µL Assay Buffer to each of 7 tubes labeled as follows: 250, 125, 62.5, 31.25, 15.625, 7.81, and 0 mU/mL GST.
4. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
5. **Use the standards within 1 hour of preparation**.



Reconstitute GST Detection Reagent

1. Allow the GST Detection Reagent to reach room temperature in the sealed bag before opening.
2. Add 300 µL of the Dry DMSO to the vial of GST Detection Reagent and vortex thoroughly.
   Note: DMSO is an aprotic organic solvent shown to enhance the absorption rate of skin-permeable substances. Wear protective gloves when using the solvent, particularly when it contains dissolved chemicals.
3. The reconstituted GST Detection Reagent is a 10X concentrate. Store any unused reconstituted GST Detection Reagent at 4°C in the desiccated pouch. **Use within 2 weeks**.

Prepare 1X GST Detection Reagent

Dilute reconstituted GST Detection Reagent 1:10 with Assay Buffer.

1. Add 150 µL of Detection Reagent to 1.35 mL of Assay Buffer for enough solution to assay half a plate.
2. Discard any excess 1X Detection Reagent.

Prepare 1X Glutathione

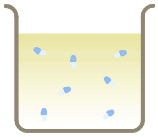
Dilute GSH 1:10 with Assay Buffer.

1. Dilute 150 µL of Glutathione with 1.35 mL of Assay Buffer for enough substrate to assay half a plate.
2. Discard any excess 1X Glutathione.

Assay procedure

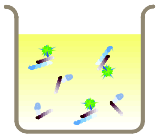
Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 30 minutes.**

IMPORTANT! Perform a standard curve with each assay.



Add sample

a. Add 50 µL of standards or diluted samples (see page 2) to the appropriate wells.



Add detection reagent and GSH

a. Add 25 µL of 1X GST Detection Reagent to each well.
b. Add 25 µL of 1X Glutathione to each well.
c. Tap the side of the plate to mix.
d. Incubate for 30 minutes at room temperature.

Read the plate and generate the standard curve

1. Read the fluorescent emission at 460 nm, with excitation at 390 nm.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background fluorescence may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the activity of unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.
   Note: Dilute samples producing signals greater than that of the highest standard in 1X Assay Buffer and reanalyze. Multiply the activity by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–500 mU/mL glutathione S-transferase.

Standard GST (mU/mL)	Mean FLU
500	35,629
250	23,147
125	14,566
62.5	9,474
31.25	6,517
15.61	5,617
7.81	4,425
0	3,669

Intra-assay precision

Four serum samples were diluted 1:2 with 1X Assay Buffer were assayed in replicates of 16 to determine precision within an assay.

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

CV = Coefficient of Variation

Inter-assay precision

Four serum samples diluted 1:2 with 1X Assay Buffer were assayed 20 times in duplicate by four operators to determine precision between assays.

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

CV = Coefficient of Variation

Performance characteristics, continued

Expected values

Random human serum and plasma (EDTA and heparin), or urine samples were tested with the assay.

Sample	Range (mU/mL)	Average (mU/mL)
Serum/plasma (n=20)	26.8–59.8	39.1
Urine (n=5)	24–102 [1]	—

[1] When normalized with the Creatinine Urinary Assay Kit (Cat. No. EIACUN), the values ranged from 2.83 –20.2 µU/mg creatinine.

Interferents

A variety of solvents and detergents were tested as possible interfering substances in the assay.

- 1% methanol or DMSO in the sample resulted in a ~10% change in GST activity.
- 0.01% Tween™ 20 or SDS in the sample resulted in no change of activity, whereas 0.01% Triton™ X-100 resulted in a >47% decrease in activity.
- Bilirubin levels of 2.5 µg/mL in the sample resulted in a < 5% decrease in GST activity.

Linearity of dilution

Linearity was determined by assaying high and low concentration lysates of Jurkat cells (high sample 10×10^6 cells/mL; low sample 0.8×10^6 cells/mL) mixed in the ratios shown in the following table.

Low Sample %	High Sample %	Expected Conc. (mU/mL)	Observed Conc. (mU/mL)	% 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	**103.4%**

Sensitivity

The analytical sensitivity of the assay is 2.70 mU/mL GST. This was determined by adding two standard deviations to the mean FLU obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Limited product warranty

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REF Catalog Number | LOT Batch code | Temperature limitation | Use by | Manufacturer | Consult instructions for use | Caution, consult accompanying documents

Manufacturer's address: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

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Corporate entity: Life Technologies Corporation | Carlsbad, CA 92008 USA | Toll Free in USA 1 800 955 6288