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ProductInformation

**Glutathione S-Transferase (GST) Assay Kit**

Catalog Number **CS0410**
Storage Temperature –20 °C

# TECHNICAL BULLETIN

## Product Description

Glutathione-S-transferases (GSTs) are a group of enzymes that are important in the detoxication of many different xenobiotics in mammals. The enzymes protect cells against toxicants by conjugating the thiol group of the glutathione to electrophilic xenobiotics, and thereby defend cells against the mutagenic, carcinogenic, and toxic effects of the compounds. GST activity was found to be present in plants,[1] insects,[2] yeast,[3] bacteria,[4] and in most mammalian tissues, especially in the liver, which plays a key role in detoxification. There are several classes of GST isozymes that differ in their specificity toward xenobiotic or endogenous substrates.

The Glutathione S-Transferase (GST) Assay Kit utilizes 1-Chloro-2,4-dinitrobenzene (CDNB) which is suitable for the broadest range of GST isozymes. Upon conjugation of the thiol group of glutathione to the CDNB substrate, there is an increase in the absorbance at 340 nm.

The Glutathione S-Transferase (GST) Assay Kit is intended for the measurement of total GST activity. It can be used to measure GST activity in cell and bacterial lysates, tissue homogenates, and in plasma and erythrocyte lysates.

## Reagents

The kit is sufficient for 500 reactions in 96 well plates or 100 assays in 1 ml cuvettes.

| Catalog Number | Description | Quantity |
|---|---|---|
| D8537 | Dulbecco's Phosphate Buffered Saline | 100 ml |
| S2444 | Sample Buffer | 5 ml |
| S2569 | Substrate (CDNB) | 1.2 ml |
| G4251 | L-Glutathione reduced | 1 g |
| G6794 | GST(control), ~0.25 mg/ml | 0.1 ml |

## Equipment and materials required but not provided

- Temperature controlled UV/visible spectrophotometer or plate reader with UV light
- Quartz cuvette 1 ml, Catalog Number S10SM
- UV 96-well plate, flat, Catalog Number CLS3635
- Water – 17 MΩ , Catalog Number W4502

## Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please refer to the Material Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices

## Preparation Instructions

**Note**: Use 17 MΩ water

1. 200 mM L-glutathione reduced
   Weigh the required amount of L-Glutathione reduced, MW 307.5, and dissolve in 17 MΩ water (e.g., 246 mg L-glutathione should be dissolved in water to make 4 ml final volume). keep the solution on ice and use it the day it is prepared. For long-term storage, the solution is stable for several months when aliquoted and stored at –20 °C. Glutathione powder can be stored at 2-8 °C.
2. GST (control) and sample
   Dilute the sample with Sample Buffer to a concentration within the activity range of the assay.

## Storage/Stability

The kit is shipped on dry ice and stored at –20 °C.

## Assay Principle[5,6,7]

GST catalyzes the conjugation of L-glutathione to CDNB through the thiol group of the glutathione.

$$\text{GSH + CDNB} \xrightarrow{\text{GST}} \text{GS-DNB Conjugate + HCl}$$

The reaction product, GS-DNB Conjugate, absorbs at 340 nm. The rate of increase in the absorption is directly proportional to the GST activity in the sample.

**Procedure**
Place the kit components on ice to thaw. Warm the Dulbecco's Phosphate Buffered Saline and the CDNB solution at 25 °C before starting the assay.

A. Assay in 1 ml quartz cuvette

1. Prepare a 10-ml reaction master mix, which is sufficient for 10 assays when a 1 ml quartz cuvette is used (Option A1). The solution must be freshly prepared for each assay series and should be used within 60 minutes of preparation.

   Alternatively, individual reactions can be prepared for 1-ml cuvettes (Option A2).

| Reagent | Option A1 | Option A2 |
|---|---|---|
| Dulbecco's Phosphate Buffered Saline | 9.8 ml | 980 µl |
| 200 mM L-Glutathione reduced | 0.1 ml | 10 µl |
| 100 mM CDNB | 0.1 ml | 10 µl |

   The solution might become slightly cloudy upon the addition of CDNB to the solution. This cloudiness disappears when the solution is completely mixed.

2. Set the spectrophotometer at 340 nm. On a kinetic program: read every 30 seconds over a period of 5 minutes after a lag time of 1 minute.

3. Transfer 1 ml of the substrate solution to a quartz cuvette and read the Blank absorbance at 340 nm

4. Add 2-50 µl GST sample or 2 µl of the GST control, provided with the kit, directly to the quartz cuvette containing up to 1 ml substrate solution. Mix by covering the cuvette with a parafilm and inverting several times.

**Note**:The sample should be read as close as possible to the addition of the GST enzyme (sample or control).

5. Record the absorbance readings according to the kinetics program described above.

**Note**: If the GST control or GST sample is too concentrated, it must be diluted with Sample Buffer prior to the assay. The dilution factor must be determined experimentally.

B. Assay in 96-well plate

1. Prepare the substrate solution as mentioned above (Section A, Option A1). A 10-ml substrate master mix is sufficient for 50 assays in 96-well plate format.

2. For analysis in 96 well plates, the concentration of the GST sample and the GST control may be too high, and should be diluted. We recommend starting with a 10 fold dilution of the GST control, e.g., 2 µl GST + 18 µl Sample Buffer.

3. Add all the components of the enzymatic reaction to a 96 well plate according to the table below. Mix well by gentle shaking for a few seconds.

We highly recommend running assays in duplicate.

| Reaction Number | 1 and 2 Control | 3 and 4 Sample | 5 and 6 Blank |
|---|---|---|---|
| Substrate solution | 196 µL | (200 –x) µL | 200 µL |
| GST, sample or control | 4 µL | x µL | _ |

x = the volume of GST sample to be assayed. It should not exceed 20 µl.

4. Read the absorbance in the plate reader at 340 nm immediately after preparing the reaction tests, and every minute thereafter to obtain at least 6 time points.

**Calculations**
The increase in absorbance is directly proportional to the GST activity. The linearity of the reaction must be determined by plotting the absorbance values against time.

Calculate the change in absorbance ($\Delta A_{340}$)/minute, in the linear range of the plot, for the sample and for the blank using the following equation:

$$(\Delta A_{340})/\text{min} = \frac{A_{340}(\text{final read}) - A_{340}(\text{initial read})}{\text{reaction time (min.)}}$$

Subtract the ($\Delta A_{340}$)/minute of the blank from the ($\Delta A_{340}$)/minute of the sample. Use this rate for the calculation of the GST specific activity.

**Note**: For measurements in 1 ml cuvettes, the initial reading is the first reading after the lag time (1 minute). Calculate the GST activity using the following equation:

GST specific activity:

$$\frac{(\Delta A_{340})/\min \times V(ml) \times dil}{\varepsilon_{mM} \times V_{enz}(ml)} = \mu mol/ml/\min$$

Where:

$dil$ = the dilution factor of the original sample

$\varepsilon_{mM}$ ($mM^{-1}cm^{-1}$) - the extinction coefficient for CDNB conjugate at 340 nm.

- for test in 1 ml cuvette = 9.6 $mM^{-1}$ (path length - 1 cm).
- for test in Sigma 96-well plate, Catalog Number CLS3635) = 5.3 $mM^{-1}$ (path length - 0.552 cm)

For a cuvette/well with a different path length, the coefficient should be calculated using these substitution factors: $\varepsilon_{mM}$ = 9.6 x path length in cm.

$V$ – the reaction volume:

- for test in 1 ml cuvette = 1 ml
- for test in 96-well plate = 0.2 ml

$V_{enz}$ **–** the volume of the enzyme sample tested

**References**


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