Glutathione S-transferase

Microplate Assay Kit

Cat #: orb390753 (manual)

Detection and Quantification of Glutathione S-transferase (GST) Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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INTRODUCTION

Glutathione S-transferases (GSTs) are a group of enzymes important in the detoxification of many 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 is present in plants, insects, yeast, bacteria, and most mammalian tissues especially in the liver, which plays a key role in detoxification.

The Glutathione S-transferase Microplate Assay Kit is based upon the GST-catalyzed reaction between GSH and the GST substrate, CDNB (1-chloro-2, 4-dinitrobenzene, which has the broadest range of isozyme detectability. Under certain conditions, the interaction between glutathione and CDNB is totally dependent on the presence of active GST. The GST-catalyzed formation of GS-DNB produces a dinitrophenyl thioether which can be detected by spectrophotometer at 340 nm.

KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Substrate I	Powder x 1	4 °C
Substrate I Diluent	1 ml x 1	4 °C
Substrate II	Powder x 1	4 °C
Positive Control	Powder x 1	4 °C
Technical Manual	1 Manual	

Note:

Substrate I: add 1 ml Substrate I Diluent to dissolve before use.

Substrate II: add 1 ml distilled water to dissolve before use.

Positive Control: add 0.2 ml Assay Buffer to dissolve before use.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 340 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times) ; centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly.

ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents in the microplate:

Reagent	Sample	Control	Positive Control
Reaction Buffer	160 µl	160 µl	160 µl
Substrate I	10 µl	10 µl	10 µl
Substrate II	10 µl	10 µl	10 µl
Sample	20 µl	--	--
Assay Buffer	--	20 µl	--
Positive Control	--	--	20 µl
Mix, measured at 340 nm and record the absorbance of 10th second and 130th second.			

Note:

1) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

CALCULATION

Unit Definition: One unit of GST activity is defined as the enzyme generates 1 µmol of GS-DNB conjugate per minute.

1. According to the protein concentration of sample

$$GST\ (U/mg) = [\ (OD_{Sample\ (130S)} - OD_{Control\ (130S)}) - (OD_{Sample\ (10S)} - OD_{Control\ (10S)})\ ] / (\varepsilon \times d) \times V_{Total} / (V_{Sample} \times C_{Protein}) / T$$

$$= 0.868 \times [\ (OD_{Sample\ (130S)} - OD_{Control\ (130S)}) - (OD_{Sample\ (10S)} - OD_{Control\ (10S)})\ ] / C_{Protein}$$

2. According to the weight of sample

$$GST\ (U/g) = [\ (OD_{Sample\ (130S)} - OD_{Control\ (130S)}) - (OD_{Sample\ (10S)} - OD_{Control\ (10S)})\ ] / (\varepsilon \times d) \times V_{Total} / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 0.868 \times [\ (OD_{Sample\ (130S)} - OD_{Control\ (130S)}) - (OD_{Sample\ (10S)} - OD_{Control\ (10S)})\ ] / W$$

3. According to the quantity of cells or bacteria

$$GST\ (U/10^4) = [\ (OD_{Sample\ (130S)} - OD_{Control\ (130S)}) - (OD_{Sample\ (10S)} - OD_{Control\ (10S)})\ ] / (\varepsilon \times d) \times V_{Total} / (V_{Sample} \times N / V_{Assay}) / T$$

$$= 0.868 \times [\ (OD_{Sample\ (130S)} - OD_{Control\ (130S)}) - (OD_{Sample\ (10S)} - OD_{Control\ (10S)})\ ] / N$$

4. According to the volume of sample

$$GST\ (U/ml) = [\ (OD_{Sample\ (130S)} - OD_{Control\ (130S)}) - (OD_{Sample\ (10S)} - OD_{Control\ (10S)})\ ] / (\varepsilon \times d) \times V_{Total} / V_{Sample} / T$$

$$= 0.868 \times [\ (OD_{Sample\ (130S)} - OD_{Control\ (130S)}) - (OD_{Sample\ (10S)} - OD_{Control\ (10S)})\ ]$$

ε: molar extinction coefficient, 9.6×10^3 L/mol/cm = 9.6 ml/µmol/cm;

d: the optical path of 96-Well microplate, 0.6 cm;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

V_{Total}: the total volume of the enzymatic reaction, 0.2 ml;

V_{Sample}: the volume of sample, 0.02 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 2 minutes.

TYPICAL DATA

Positive Control reaction in 96-well plate assay with decreasing the concentration