Glutathione S-transferase Activity Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1047

(Version 1.2D)

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.

I. 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.

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.

II.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer	20 mlx 1	4 °C
SubstrateI	Powder x 1	4 °C
SubstrateIDiluent	1 mlx 1	4 °C
SubstrateII	Powder x 1	4 °C
Positive Control	Powder x 1	4 °C
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III. 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

IV. REAGENT PREPARATION

SubstrateI: Briefly centrifuge prior to opening.Add 1 ml SubstrateIDiluent to dissolve before use.Keep in dark and store at 4 °C for 1-2 months or -20°C for 3-4months after reconstitution.

SubstrateII: Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve before use.Keep in dark for immediate use or store at -20°C for 1 week after reconstitution.

Positive Control: Briefly centrifuge prior to opening. Add 0.2 ml Assay Buffer to dissolve before use.Store at -80°C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay 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 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay Buffer on ice, centrifuged at 10000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For serum or plasma samples

Detect directly.

VI. 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
SubstrateI	10 µl	10 µl	10 µl
SubstrateII	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:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

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

1.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(OD_{Sample(130)} - OD_{Sample(10)}) - (OD_{Control(130)} - OD_{Control(10)})}{\varepsilon \times d \times C_{Protein} \times T} \times \frac{V_{Total}}{V_{Sample}} (U/mg)$$

1.2 According to the weight of sample

$$\text{Activity} = \frac{(OD_{Sample(130)} - OD_{Sample(10)}) - (OD_{Control(130)} - OD_{Control(10)})}{\varepsilon \times d \times W \times T} \times \frac{V_{Total} \times V_{Assay}}{V_{Sample}} (U/g)$$

1.3According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(OD_{Sample(130)} - OD_{Sample(10)}) - (OD_{Control(130)} - OD_{Control(10)})}{\varepsilon \times d \times N \times T} \times \frac{V_{Total} \times V_{Assay}}{V_{Sample}} (U/10^4)$$

1.4 According to the volume of sample

$$\text{Activity} = \frac{(OD_{Sample(130)} - OD_{Sample(10)}) - (OD_{Control(130)} - OD_{Control(10)})}{\varepsilon \times d \times T} \times \frac{V_{Total}}{V_{Sample}} (U/mg)$$

ε: molar extinction coefficient,9.6× 10^3L/mol/cm = 9.6ml/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 ×10^4

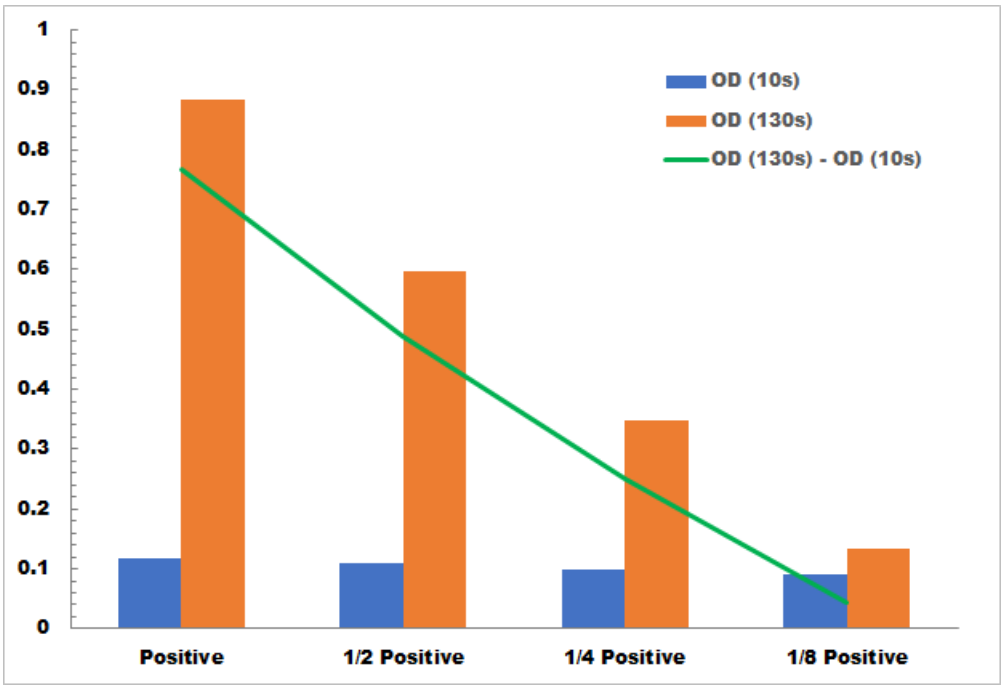
V_{Total}: thetotal volume of all reagents in assay procedure, ml

V_{Sample}: the volume of sample in assay procedure, ml

V_{Assay}: the volume of Assay Buffer II in sample preparation, ml

T: the reaction time, minutes

VIII. TYPICAL DATA



Positive Control reaction in 96-well plate assay with decreasing theconcentration