



Glutathione S-transferase Microplate Assay Kit User Manual

Catalog # ASK1047

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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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.

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.



II. 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
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Note:

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

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

III. MATERIALS REQUIRED BUT NOT PROVIDED

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



IV. 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%, sonication 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.



V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents in the microplate:

Reagent	Sample	Blank
Reaction Buffer	160 μ l	160 μ l
Substrate I	10 μ l	10 μ l
Substrate II	10 μ l	10 μ l
Sample	20 μ l	--
Assay Buffer	--	20 μ l

Mix, measured at 340 nm and record the absorbance of 10th second and 130th second.

VI. 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

$$\begin{aligned}\text{GST (U/mg)} &= [(OD_{\text{Sample}(130\text{S})} - OD_{\text{Control}(130\text{S})}) - (OD_{\text{Sample}(10\text{S})} - OD_{\text{Control}(10\text{S})})] / (\epsilon \times d) \times \\ &\quad V_{\text{Total}} / (V_{\text{Sample}} \times C_{\text{Protein}}) / T \\ &= 0.868 \times [(OD_{\text{Sample}(130\text{S})} - OD_{\text{Control}(130\text{S})}) - (OD_{\text{Sample}(10\text{S})} - OD_{\text{Control}(10\text{S})})] / \\ &\quad C_{\text{Protein}}\end{aligned}$$

2. According to the weight of sample

$$\begin{aligned}\text{GST (U/g)} &= [(OD_{\text{Sample}(130\text{S})} - OD_{\text{Control}(130\text{S})}) - (OD_{\text{Sample}(10\text{S})} - OD_{\text{Control}(10\text{S})})] / (\epsilon \times d) \times V_{\text{Total}} \\ &\quad / (V_{\text{Sample}} \times W / V_{\text{Assay}}) / T \\ &= 0.868 \times [(OD_{\text{Sample}(130\text{S})} - OD_{\text{Control}(130\text{S})}) - (OD_{\text{Sample}(10\text{S})} - OD_{\text{Control}(10\text{S})})] / W\end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned}\text{GST (U}/10^4) &= [(OD_{\text{Sample}(130\text{S})} - OD_{\text{Control}(130\text{S})}) - (OD_{\text{Sample}(10\text{S})} - OD_{\text{Control}(10\text{S})})] / (\epsilon \times d) \times \\ &\quad V_{\text{Total}} / (V_{\text{Sample}} \times N / V_{\text{Assay}}) / T \\ &= 0.868 \times [(OD_{\text{Sample}(130\text{S})} - OD_{\text{Control}(130\text{S})}) - (OD_{\text{Sample}(10\text{S})} - OD_{\text{Control}(10\text{S})})] / N\end{aligned}$$

4. According to the volume of sample

$$\begin{aligned}\text{GST (U/ml)} &= [(OD_{\text{Sample}(130\text{S})} - OD_{\text{Control}(130\text{S})}) - (OD_{\text{Sample}(10\text{S})} - OD_{\text{Control}(10\text{S})})] / (\epsilon \times d) \times \\ &\quad V_{\text{Total}} / V_{\text{Sample}} / T \\ &= 0.868 \times [(OD_{\text{Sample}(130\text{S})} - OD_{\text{Control}(130\text{S})}) - (OD_{\text{Sample}(10\text{S})} - OD_{\text{Control}(10\text{S})})]\end{aligned}$$

ϵ : molar extinction coefficient, $9.6 \times 10^3 \text{ L/mol/cm} = 9.6 \text{ ml}/\mu\text{mol/cm}$;

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.