Gamma-glutamyltransferase

Microplate Assay Kit

User Manual

Catalog # CAK1157

(Version 1.3B)

Detection and Quantification of Gamma-glutamyltransferase (GGT) Content in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, Other biological fluids Samples.

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

I. INTRODUCTION

Gamma-glutamyltransferase (also γ-glutamyltransferase, GGT, gamma-GT; EC 2.3.2.2) is a transferase (a type of enzyme) that catalyzes the transfer of gamma-glutamyl functional groups from molecules such as glutathione to an acceptor that may be an amino acid, a peptide or water (forming glutamate). GGT plays a key role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione and drug and xenobiotic detoxification. Other lines of evidence indicate that GGT can also exert a pro-oxidant role, with regulatory effects at various levels in cellular signal transduction and cellular pathophysiology. This transferase is found in many tissues, the most notable one being the liver, and has significance in medicine as a diagnostic marker.

Gamma-glutamyltransferase Microplate Assay Kit is a sensitive assay for determining Gamma-glutamyltransferase activity in various samples. Gamma-glutamyltransferase activity is determined by the product of paranitroaniline. The reaction products can be measured at a colorimetric readout at 405 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate I	Powder x 1	4 °C
Substrate I Diluent	10 ml x 1	4 °C
Substrate II	Powder x 1	4 °C
Reaction Buffer	1.5 ml x 1	4 °C
Stop Solution	10 ml x 1	4 °C
Standard (5 µmol/ml)	1 ml x 1	4 °C
Positive Control	Powder x 1	-20 °C
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Note:

Substrate I: add 10 ml Substrate I Diluent to dissolve before use; store at -20 °C for a month after reconstitution.

Substrate II: add 1 ml Reaction Buffer to dissolve before use; store at -20 °C for a month after reconstitution.

Positive Control: add 100 µl Assay Buffer to dissolve before use; store at -80 °C for a month after reconstitution.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 405 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

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%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 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 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum, plasma or other liquid samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Sample	10 µl	--	--	--	--
Standard	--	--	10 µl	--	--
Positive Control	--	--	--	--	10 µl
Reaction Buffer	--	--	10 µl	10 µl	--
Distilled water	--	10 µl	--	10 µl	--
Substrate I	100 µl	100 µl	100 µl	100 µl	100 µl
Shake and mix, put it into the oven, 37 °C for 5 minutes.					
Substrate II	10 µl	10 µl	--	--	10 µl
Shake and mix, put it into the oven, 37 °C for 10 minutes.					
Stop Solution	100 µl	100 µl	100 µl	100 µl	100 µl
Record absorbance measured at 405 nm.					

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of GGT activity is defined as the enzyme generates 1 μmol paranitroaniline per minute.

1. According to the protein concentration of sample

$$GGT\ (U/mg) = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 0.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$GGT\ (U/g) = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 0.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$GGT\ (U/10^4) = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

$$= 0.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

$C_{Standard}$: the standard concentration, 5 μmol/ml;

$V_{Standard}$: the volume of standard, 0.01 ml;

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

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

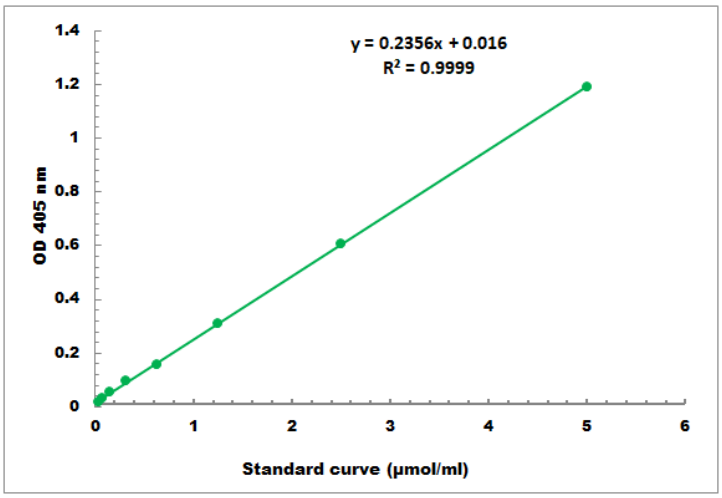
V_{Sample}: the volume of sample, 0.01 ml;

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

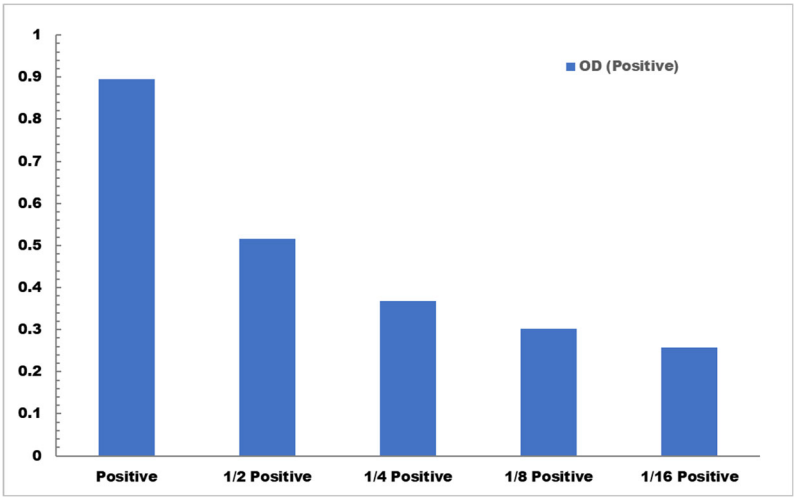
T: the reaction time, 10 minutes.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.01 µmol/ml - 5 µmol/ml



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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES