



## CheKine™ Micro Gamma-Glutamyl Transpeptidase (GGT) Activity Assay Kit

Cat #: KTB1690

Size: 96 T/96 S

	<b>Micro Gamma-Glutamyl Transpeptidase (GGT) Activity Assay Kit</b>		
<b>REF</b>	<b>Cat #:</b> KTB1690	<b>LOT</b>	<b>Lot #:</b> Refer to product label
	<b>Applicable samples:</b> Animal and Plant Tissues, Cells, Bacteria,		
	<b>Storage:</b> Stored at 4°C for 6 months		

### Assay Principle

Gamma-Glutamyl Transpeptidase (GGT) is a key enzyme in the  $\gamma$ -glutamyl cycle and catalyzes the degradation of GSH. GGT catalyzes the transfer of the  $\gamma$ -glutamyl group on GSH or other  $\gamma$ -glutamyl compounds to the receptor, and can also catalyze the hydrolysis of GSH and other  $\gamma$ -glutamyl compounds to produce glutamate, and is outside the cell. Glutathione plays an important role in the metabolism. Gamma-Glutamyl Transpeptidase (GGT) catalyzes the transfer of  $\gamma$ -glutamyl group in glutamyl p-nitroaniline to N-glycylglycine to generate p-nitroaniline, which has characteristic light absorption at 405 nm; calculate  $\gamma$ -GT enzyme activity by measuring the increase rate of light absorption at 405 nm.

### Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
	96 T	
Extraction Buffer	120 mL	4°C
Reagent I	Powder×1 vial	4°C
Reagent II	5 mL	4°C
Reagent III	18.5 mL	4°C

**Note:** Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

### Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 405 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Refrigerated centrifuge, water bath, ice maker, incubator
- Deionized water
- Homogenizer (for tissue samples)

### Reagent Preparation

**Extraction Buffer:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

**Working Reagent:** Prepare before use, dilute Reagent I with Reagent II and fully dissolve it (when the room temperature is too low, it can be dissolved in a 40°C incubator); Then, add Reagent III to the bottle containing Reagent I, mix well. The prepared reagent can be stored at 4°C, protected from light for 1 month.

**Note: Reagent I is toxic, so it is recommended to experiment in a fume hood.**

## Sample Preparation

**Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80°C for one month. Sample processing and other processes need to be carried out on ice, and the enzyme activity must be determined on the same day to avoid affecting its activity. If it is a homogenate, avoid repeated freezing and thawing. When measuring GGT activity in cells, the number of cells must be between 3 million and 5 million. When extracting GGT from cells, Extraction Buffer can be added for grinding or ultrasonic treatment. Cells cannot be treated with cell lysate (prevent enzyme inactivation due to protein denaturation).**

1. Tissue samples: Weigh about 0.1 g tissue and add 1 mL Extraction Buffer. Homogenize on ice. Centrifuge at 8,000 g for 15 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

2. Bacteria, cells: Collect  $5 \times 10^6$  bacteria or cells into the centrifuge tube, add 1 mL Extraction Buffer to ultrasonically disrupt the bacteria or cells 3 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s). Centrifuge at 8,000 g for 15 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

**Note: It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.**

## Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 405 nm, visible spectrophotometer was returned to zero with deionized water.

2. Place Working Reagent in incubator at 37°C (mammals) or 25°C (other species) for 30 min (ensure no precipitation in Working Reagent).

3. Add the following reagents respectively.

Reagent	Blank Well (μL)	Test Well (μL)
Sample	0	20
Deionized Water	20	0
Working Reagent	180	180

Mix well, the absorbance values at 405 nm of 10 s and 70 s are measured. The values of blank well recorded as  $A_1$ ,  $A_2$ , test well recorded as  $A_3$ ,  $A_4$ , respectively. Finally, calculate  $\Delta A_{\text{Test}} = (A_4 - A_3) - (A_2 - A_1)$ .

## Data Analysis

**Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.**

A. 96-well plates calculation formula as below

1. Calculated by protein concentration

Active unit definition: One unit of GGT enzyme is defined as 1 μmol of p-nitroaniline is catalyzed by one mg of protein per min at 25°C or 37°C.

$$\text{GGT (U/mg prot)} = [\Delta A_{\text{Test}} \times V_{\text{Total}} \div (\epsilon \times d) \times 10^6] \div (\text{Cpr} \times V_{\text{Sample}}) \div T = \mathbf{2.026 \times \Delta A_{\text{Test}} \div \text{Cpr}}$$

2. Calculated by fresh weight of tissues

Active unit definition: One unit of GGT enzyme is defined as 1 μmol of p-nitroaniline is catalyzed by one g of tissue per min at 25°C or 37°C.

$$\text{GGT (U/g fresh weight)} = [\Delta A_{\text{Test}} \times V_{\text{Total}} \div (\epsilon \times d) \times 10^6] \div (W \div V_{\text{Sample Total}} \times V_{\text{Sample}}) \div T = 2.026 \times \Delta A_{\text{Test}} \div W$$

### 3. Calculated by bacteria or cells samples

Active unit definition: One unit of GGT enzyme is defined as 1 μmol of p-nitroaniline is catalyzed by 10<sup>4</sup> bacteria or cells.

$$\text{GGT (U/10}^4\text{)} = [\Delta A_{\text{Test}} \times V_{\text{Total}} \div (\epsilon \times d) \times 10^6] \div (500 \times V_{\text{Sample}} \div V_{\text{Sample Total}}) \div T = 4.053 \times 10^{-3} \times \Delta A_{\text{Test}}$$

Where: V<sub>Sample</sub>: sample volume added, 0.02 mL; V<sub>Sample Total</sub>: extract buffer added to samples, 1 mL; V<sub>Total</sub>: total reaction volume, 2×10<sup>-4</sup> L; T: reaction time, 1 min; Cpr: sample protein concentration, mg/mL; W: sample weight, g; 500: Total number of bacteria or cells, 5×10<sup>6</sup>; ε: the extinction coefficient of p-nitroaniline, 9,870 L/mol/cm; d: optical path of cuvette, 0.5 cm; 10<sup>6</sup>: 1 mol=10<sup>6</sup> μmol.

### B. Microglass cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

## Recommended Products

Catalog No.	Product Name
KTB1600	CheKine™ Micro Reduced Glutathione (GSH) Assay Kit
KTB1610	CheKine™ Micro Glutathione Oxidized (GSSG) Assay Ki
KTB1620	CheKine™ Micro Glutathione Reductases (GR) Activity Assay Kit
KTB1630	CheKine™ Micro Glutathione S-Transferase (GST) Activity Assay Kit

## Disclaimer

The reagent is only use in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.