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

Cat #: KTB1690 Size: 96 T

| | | | |
|---|---|---|---|
| | **Micro Gamma-Glutamyl Transpeptidase (GGT) Activity Assay Kit** | | |
| REF | **Cat #:** KTB1690 | LOT | **Lot #:** Refer to product label |
| | **Applicable samples:** Serum, Plasma, Animal and Plant Tissues, Cells, Bacteria, | | |
| | **Storage:** Stored at 4℃ for 6 months | | |

## Assay Principle

Gamma-Glutamyl Transpeptidase (GGT) is a key enzyme in the γ-glutamyl cycle and catalyzes the degradation of GSH. GGT catalyzes the transfer of the γ-glutamyl group on GSH or other γ-glutamyl compounds to the receptor, and can also catalyze the hydrolysis of GSH and other γ-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 γ-glutamyl group in glutamyl p-nitroaniline to N-glycylglycine to generate p-nitroaniline, which has characteristic light absorption at 405 nm; calculate γ-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 | 100 mL | 4℃ |
| Reagent Ⅰ | 1 | 4℃ |
| Reagent Ⅱ | 4 mL | 4℃ |
| Reagent Ⅲ | 14.8 mL | 4℃ |

## 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℃.

**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℃ incubator); Then, add Reagent Ⅲ to the bottle containing Reagent Ⅰ, mix well. Store at 4℃.

## Sample Preparation

**Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ 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℃. Use supernatant for assay, and place it on ice to be tested.

2. Bacteria, cells: Collect $5\times10^6$ 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℃. Use supernatant for assay, and place it on ice to be tested.

3. Serum and other liquid samples: Tested directly.

**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℃ (mammals) or 25℃ (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 $\Delta A_{Test}=(A_4-A_3)-(A_2-A_1)$.

## 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℃ or 37℃.

GGT (U/mg prot)=$[\Delta A_{Test}\times V_{Total}\div(\varepsilon\times d)\times10^6]\div(Cpr\times V_{Sample})\div T$**=$2.026\times\Delta A_{Test}\div Cpr$**

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℃ or 37℃.

GGT (U/g fresh weight)=$[\Delta A_{Test}\times V_{Total}\div(\varepsilon\times d)\times10^6]\div(W\div V_{Sample\ Total}\times V_{Sample})\div T$**=$2.026\times\Delta A_{Test}\div W$**

3. Calculated by serum or plasma

Active unit definition: One unit of GGT enzyme is defined as 1 μmol of p-nitroaniline is catalyzed by 1 mL serum or plasma per min at 25℃ or 37℃.

GGT (U/mL)=[$\Delta A_{Test}$×$V_{Total}$÷(ε×d)×$10^6$]÷$V_{Sample}$÷T**=2.026×$\Delta A_{Test}$**

4. 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 $10^4$ bacteria or cells.

GGT (U/$10^4$)=[$\Delta A_{Test}$×$V_{Total}$÷(ε×d)×$10^6$]÷(500×$V_{Sample}$÷$V_{Sample\ Total}$)÷T**=4.053×$10^{-3}$×$\Delta A_{Test}$**

Where: $V_{Sample}$: sample volume added,0.02 mL; $V_{Sample\ Total}$: extract buffer added to samples, 1 mL;$V_{Total}$: total reaction volume. 2×$10^{-4}$ L; T: reaction time, 1 min; Cpr: sample protein concentration, mg/mL; W: sample weight, g; $V_{Serum\ (Plasma)}$: the volume of serum (plasma), 0.02 mL; 500: Total number of bacteria or cells, 5×$10^6$; ε: the extinction coefficient of p-nitroaniline, 9,870 L/mol/cm; d: optical path of cuvette, 0.5 cm; $10^6$:1 mol=$10^6$ 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.