

# **$\gamma$ -GlutamylTranspeptidase ( $\gamma$ -GT) Activity Assay Kit**

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer/microplate reader

**Catalog Number:** NA0536

**Size:** 100T/96S

## **Components**

Extract: 100 mL $\times$ 1. Storage at 4 °C.

Reagent I: Powder $\times$ 1. Storage at 4°C.

Reagent II:4.9 mL $\times$ 1. Storage at 4°C.

Reagent III: 18.4 mL $\times$ 1. Storage at 4°C.

Working solution (prepare in Reagent I bottle): prepare when the solution will be used, pour the Reagent II into Reagent I bottle, fully dissolved (incubate in 40°C water bath to promote the dissolution if the room temperature is too low). Then pour Reagent III into Reagent I bottle, mix well and store at room temperature.

## **Product Description**

$\gamma$ -glutamyltranspeptidase ( $\gamma$ -GT) is a key enzyme in  $\gamma$ -glutanyl cycle, which catalyzes the degradation of GSH.  $\gamma$ -GT catalyzes the transfer of  $\gamma$ -glutamyl groups from GSH or other  $\gamma$ -glutamyl compounds to receptors. It can also catalyze the hydrolysis of GSH and other  $\gamma$ -glutamyl compounds to produce glutamate, which plays an important role in the metabolism of extracellular glutathione.

$\gamma$ -GT catalyzes the transfer of  $\gamma$ -glutamyl in glutamyl p-nitroaniline to N-glycylglycine to form p-nitroaniline with characteristic light absorption at 405 nm.  $\gamma$ -GT enzyme activity was calculated by measuring the increase rate of light absorption at 405 nm.

## **Reagents and Equipment Required but Not Provided.**

Spectrophotometer/Microplate reader,ultra-micro glass cuvette/96 well flat-bottom plate, low temperature centrifuge, water-bath, adjustable pipette, ice, mortar/homogenizer and distilled water.

## **Procedure**

### **I. Extraction of crude enzyme solution:**

1. Bacteria or cultured cells:

Collect bacteria or cells into centrifuge tube, discard supernatant after centrifugation; the number of bacteria or cells ( $10^4$ ): the Extract solution volume (mL) is 500~1000:1 (it is recommended that add 1 mL of the extract solution to 5 million bacteria or cells), and break the bacteria or cells by ultrasound (ice bath, 20% power or 200W, ultrasound 3s, interval of 10s, repeat for 30 times). Centrifuge at 10000 rpm for 10 minutes at 4°C, take the supernatant and place it on ice for test.

2. Tissue:

Weigh about 0.1 g of sample, add 1.0 mL of Extract solution, full grinding. Centrifuge at 10000 rpm for 15 minutes at 4°C, take the supernatant and place it on ice for test.

3. Serum (plasma): Direct detection.

### 1. Test Steps:

- 1) Preheat the Spectrophotometer/Microplate reader for more than 30 minutes, adjust the wavelength to 405 nm and set the zero with distilled water.
- 2) Place working solution at 25°C (general species) or 37°C (mammals) water bath, preheating for more than 30 minutes (Ensure that there is no precipitation).
- 3) Sample test:

Reagent (μL)	Blank Tube (A <sub>B</sub> )	Test tube (A <sub>T</sub> )
Distilled water	20	-
Supernatant/serum	-	20
Working solution	180	180

After mixing thoroughly, detect the absorbance value at 405 nm at 10s (A<sub>1</sub>) and 130s (A<sub>2</sub>), Calculation:  $\Delta A = A_2 - A_1$ . Calculate  $\Delta A_T = \Delta A - \Delta A_B$ .

## III. Calculation of $\gamma$ -GT activity

### A. Calculate by 96 well flat-bottom plate

1. Calculate by sample protein concentration

Active unit (U) definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the produce of 1 μmol of P-nitroaniline per minute at 25°C or 37°C every milligram of protein.

$$\gamma\text{-GT (U/mg prot)} = \Delta A_T \div (\epsilon \times d) \times 10^6 \times V_{TV} \div (C_{pr} \times V_S) \div T = 0.845 \times \Delta A_T \div C_{pr}$$

2. Calculate by sample fresh weight

Active unit (U) definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the produce of 1 μmol of P-nitroaniline per minute at 25°C or 37°C every gram of tissue.

$$\gamma\text{-GT (U/g fresh weight)} = \Delta A_T \div (\epsilon \times d) \times 10^6 \times V_{TV} \div (W \div V_E \times V_S) \div T = 0.845 \times \Delta A_T \div W$$

3. Calculate by serum (plasma)

Active unit (U) definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the produce of 1 μmol of P-nitroaniline per minute at 25°C or 37°C every per liter of serum.

$$\gamma\text{-GT (U/L serum (plasma))} = \Delta A_T \div (\epsilon \times d) \times 10^6 \times V_{se(pla)} \div T = 0.845 \times \Delta A_T$$

4. Calculated by bacteria or cultured cells

Active unit (U) definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the produce of 1 μmol of P-nitroaniline per minute at 25°C or 37°C every ten thousand bacteria or cells.

$$\gamma\text{-GT (U/10}^4\text{cell)} = \Delta A_T \div (\epsilon \times d) \times 10^6 \div (500 \times V_S \div V_E) \div T = 1.69 \times 10^{-3} \times \Delta A_T$$

V<sub>S</sub>: Add sample volume, 0.02 mL;

V<sub>E</sub>: Add extraction liquid volume: 1 mL;

T: Reaction time, 2 minutes;

C<sub>pr</sub>: Sample protein concentration, mg/mL;

W: Sample weight, g;

5 million: 5 million cells;

$\epsilon$ : The extinction coefficient of P-nitroaniline is 9870 L/mol/cm;

d: Light path of cuvette, 0.6 cm;

$V_{TV}$ : Total volume of reaction system,  $2 \times 10^{-4}$  L;

$10^6$ : Unit conversion coefficient,  $1 \text{ mol} = 10^6 \mu\text{mol}$ ;

$V_{se(pla)}$ : Volume of serum (plasma), 0.02 mL.

### **B. Calculate by the micro-glass cuvette**

Change the  $d = 0.6$  cm in the above calculation formula to  $d = 1$  cm (light path of 96-well plate)

#### **Note:**

When measure the activity of  $\gamma$ -GT in cultured cells, the extraction process of  $\gamma$ -GT in cells could by grinding or ultrasonic treatment after adding reagents. Cells can not treat with cell lysis buffer (prevent the deactivation of enzymes due to protein degeneration).

#### **Experimental instances:**

1. Take 0.1g of kidney, add 1mL of extract solution, homogenate and grind. Centrifuge at 10000rpm for 15 minutes at  $4^\circ\text{C}$ , take the supernatant, dilute it by 4 times, and test according to the measured steps. Calculate  $\Delta A_T = A_{T2} - A_{T1} = 2.088 - 0.638 = 1.45$ ,  $\Delta A_B = A_{B2} - A_{B1} = 0.435 - 0.425 = 0.01$ ,  $\Delta A = \Delta A_T - \Delta A_B = 1.45 - 0.01 = 1.44$ , calculate the enzyme activity according to sample weight:

$\gamma$ -GT (U/g weight) =  $0.845 \times \Delta A \div W \times 4$  (Dilution Ratio) = 48.67 U/g weight.

#### **Related products:**

NA0781/ NA0540    Reduced Glutathione (GSH) Assay Kit

NA0780/NA0539    Oxidized Glutathione (GSSG) Assay Kit