γ-GlutamylTranspeptidase (γ-GT) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: BC1225

Size: 100T/96S

Components

Extract: 100 mL×1. Storage at 4 ℃.

Reagent I: Powder×1. Storage at 4℃.

Reagent II:4.9 mL×1. Storage at 4℃.

Reagent III: 18.4 mL×1. Storage at 4℃.

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℃ 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

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

γ-GT catalyzes the transfer of γ-glutamyl in glutamyl p-nitroaniline to N-glycylglycine to form p-nitroaniline with characteristic light absorption at 405 nm. γ-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℃, 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℃, 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℃ (general species) or 37℃ (mammals) water bath, preheating for more than 30 minutes (Ensure that there is no precipitation).

3) Sample test:

Reagent (μL)	Blank Tube (A_B)	Test tube (A_T)
Distilled water	20	-
Supernatant/serum	-	20
Working solution	180	180

After mixing thoroughly, detect the absorbance value at 405 nm at 10s (A1) and 130s (A2), Calculation: ΔA = A2-A1. Calculate $\Delta A_T = \Delta A - \Delta A_B$.

III. Calculation of γ-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-nitroanilineper minute at 25℃ or 37℃every milligram of protein.

γ-GT (U/mg prot)=$\Delta A_T \div (\varepsilon \times d) \times 10^6 \times V_{TV} \div (Cpr \times V_S) \div T = 0.845 \times \Delta A_T \div Cpr$.

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℃ or 37℃ every gram of tissue.

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

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℃ or 37℃ every per liter of serum.

γ-GT (U/L serum (plasma)=$\Delta A_T \div (\varepsilon \times d) \times 10^6 \times Vse(pla) \div T = 0.845 \times \Delta A_T$.

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℃ or 37℃ every ten thousand bacteria or cells.

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

V_S: Add sample volume, 0.02 mL;

V_E: Add extraction liquid volume: 1 mL;

T: Reaction time, 2 minutes;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

5 million: 5 million cells;

ε: 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, 2×10^{-4} L;

10^6: Unit conversion coefficient, 1mol=10^6 μmol;

Vse(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= 1cm (light path of 96-well plate)

Note:

When measure the activity of γ-GT in cultured cells, the extraction process of γ-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℃, 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:

γ-GT（U/g weight）=0.845×ΔA÷W×4（Dilution Ratio）=48.67 U/g weight.

Related products：

BC1170/ BC1175	Reduced Glutathione (GSH) Assay Kit
BC1180/BC1185	Oxidized Glutathione (GSSG) Assay Kit