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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: AK0469

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 ofP-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 ofP-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:

AK0478/ AK0477	Reduced Glutathione (GSH) Assay Kit
AK0476/AK0475	Oxidized Glutathione (GSSG) Assay Kit