γ-GlutamylTranspeptidase (γ-GT) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: NA0777

Size: 50T/48S

Components

Extract solution:50 mL×1.Storage at 4℃.

Reagent I: Powder×1. Storage at 4℃.

ReagentII:12.5mL×1. Storage at 4℃.

ReagentIII: 44.5mL×1. Storage at 4℃.

Working solution (prepare in Reagent I bottle): prepare when the solution will be used, pour the ReagentII into Reagent I bottle, fully dissolved (incubate in 40℃ water bath to promote the dissolution if the room temperature is too low). Then pour ReagentIII 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 andother 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 is calculated by measuring the increase rate of light absorption at 405nm.

Reagents and Equipment Required but Not Provided

Spectrophotometer, centrifuge, water-bath,adjustable pipette, 1mL glass cuvette, 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 the supernatant after centrifugation. According to the number of bacteria or cells (10^4): the Extract solution volume (mL) is 500~1000:1 (it is recommended that add 1mL of the Extract solution to 5 million bacteria or cells), break the bacteria or cells by ultrasound (ice bath, 20% power or 200W, ultrasound 3s, interval of 10s, repeat for 30 times).Centrifuge at 10000rpm for 10 minutes at 4℃, take the supernatant and place it on ice for test.

2. Tissue:

Weigh about 0.1 g of samples, add 1.0 mL of extract solution, full grinding.Centrifuge at 10000rpmfor 15 minutes at 4℃, take the supernatant and place it on ice for test.

3. Serum (plasma):

Direct detection.

II. Test Steps：

1) Preheat the Spectrophotometer for more than 30minutes, adjust the wavelength to 405nm 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	100	-
Supernatant/serum	-	100
Working solution	900	900

After mixing thoroughly, detect the absorbance valueat 405nm 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

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 μmolof P-nitroanilineper minute at 25℃ or 37℃ every milligram of protein.

$\gamma\text{-GT(U/mg prot)}=\Delta A_T \div(\varepsilon\times d)\times 10^6\times V_{TV}\div(Cpr\times V_S)\div T=0.506\times\Delta A_T\div Cpr$.

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 μmolof P-nitroaniline per minute at 25℃ or 37℃every gram of tissue.

$\gamma\text{-GT(U/g fresh weight)}=\Delta A_T \div(\varepsilon\times d)\times 10^6\times V_{TV}\div(W\div V_E\times V_S)\div T=0.506\times\Delta A_T\div W$.

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 μmolof P-nitroaniline per minute at 25℃ or 37℃every per liter of serum.

$\gamma\text{-GT(U/L serum (plasma)}=\Delta A_T \div(\varepsilon\times d)\times 10^6\times Vse(pla)\div T=0.506\times\Delta A_T$.

4. Calculated by bacteria or cultured cells

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

$\gamma\text{-GT(U/10}^4\text{ cell)}=\Delta A_T \div(\varepsilon\times d)\times 10^6\div(500\times V_S\div V_E)\div T=0.001\times\Delta A_T$.

V_S: Add sample volume, 0.1mL;

V_E: Add extraction liquid volume: 1mL;

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, 1cm;
V_{TV}: Total volume of reaction system, 0.001L;
10^6: Unit conversion coefficient, 1mol=10^6μmol;
Vse(pla): Volume of serum (plasma), 0.1mL.

Note:

When measure the activity of γ-GT in cultured cells, the extraction processof γ-GT in cells could by grinding or ultrasonic treatment after adding reagentI.Cells can not treat with cell lysis buffer (prevent the deactivation of enzymesdue to protein degeneration).

Experimental instances：

1. Take 0.1g of kidney, add 1mL of extract solution, homogenate and grind. Centrifuge at 10000rpm for 10 minutes at 4℃, take the supernatant, dilute it by 20 times, and test according to the measured steps. Calculate $\Delta A_T=A_{T2}-A_{T1}=1.412-0.68=0.732$， $\Delta A_B= A_{B2}- A_{B1}=0.597-0.578=0.019$, calculate the enzyme activity according to sample weight:

γ-GT（U/g weight）=0.506×ΔA÷W×20（dilution ratio）=72.16 U/g weight.

Related products：

NA0781/ NA0540	Reduced Glutathione (GSH) Assay Kit
NA0780/NA0539	Oxidized Glutathione (GSSG) Assay Kit