BT LAB
Bioassay Technology Laboratory
Optimize Your Research

Alanine Aminotransferase (ALT/GPT) Assay Kit

Catalog No: SH0388
Method: Colorimetric method
Specification: 100 tubes/50 samples

Application

This kit can be used to measure ALT/GPT activity in serum, plasma, tissue, culture supernatant and other samples.

Experimental instruments

Test tube, Micropipettor, 37℃water bath, Vortex mixer, Microplate reader (510 nm).

Detection principle

Alanine Aminotransferase (ALT/GPT) catalyze alanine and α-ketoglutaric acid to generate pyroracemic acid and glutamic acid at 37 ℃ and pH7.4. after reaction for 30 mins, Then add 2,4-dinitrophenylhydrazine(DNPH) hydrochloric
acid solution to stop the reaction and generate phenylhydrazone pyruvate simultaneously.
Phenylhydrazone appears reddish brown in alkaline condition, measure the OD value and calculate the ALT/GPT activity indirectly.

Kit components

Reagent 1: ALT substrate solution, 5 mL ×1 vial. Store at 4℃ for 6 months.
Reagent 2: DNPH solution, 5 mL ×1 vial. Store at 4℃ for 6 months.
Reagent 3: 4 mol/L NaOH solution, 5 mL ×1 vial. Sealed at room temperature for 6 months.
Preparation of 0.4 mol/L NaOH solution: Dilute the 4 mol/L NaOH solution with double-distilled water as 1:9 before use. Prepared as much as you need. Store at room temperature.
Reagent 4: 2 μmol/mL Sodium pyruvate standard solution, 1 mL ×1 vial. Store at 4℃ for 6 months.
Reagent 5: 0.1 mol/L Phosphate buffer, 1 mL ×1 vial. Store at 4℃ for 6 months.

Sample preparation

1. **Serum (plasma) and other liquid sample:** Detect the sample directly.
2. **Animal tissue sample:** Accurately weigh the tissue sample, add 9 times the volume of normal saline according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 2500 rpm for 10 min, then take the supernatant for detection (Take a small part of supernatant for detecting the protein concentration with CBB or BCA).
3. **Culture cell sample:** Wash the cells with iso-osmia buffer (0.1 mol/L, pH7~7.4 phosphate buffer or normal saline is recommended) 1~2 times. Centrifuge at 1000 rpm for 10 min and then remove the supernatant and keep the cell sediment. Add homogenate media (0.1 mol/L, pH7~7.4 phosphate buffer or normal saline). Sonicate or grind with hand-operated in ice water bath. Prepared the homogenate liquid without centrifugation.

Operation steps

	Sample tube	Control tube
Substrate solution (μL), pre-heated at 37℃	20	20
Sample (μL)	5	
Mix fully and incubate at 37℃ for 30 min accurately.		
	Sample tube	Control tube
DNPH solution (μL)	20	20
Sample (μL)		5
Mix fully and incubate at 37℃ for 20 min accurately.		
0.4 mol/L NAOH (μL)	200	200
Gently shake the microplate to mix thoroughly. Stand for 15 min at room temperature. Measure the OD values at 510 nm.		

Calculate the corresponding ALT/GPT activity according to the standard curve with the ΔOD ($\Delta OD = OD_{Sample} - OD_{Control}$).

Calculation of results

1. **Serum/plasma:**
   ALT/GPT activity (U/L)
   =ALT activity calculated from standard curve(U/L) × Dilution multiple
2. **Tissue and Cells:**
   ALT/GPT activity (U/*gprot*)
   =ALT activity calculated from standard curve (U/L)
   ÷ Concentration of the protein tested (*gprot*)

Notes

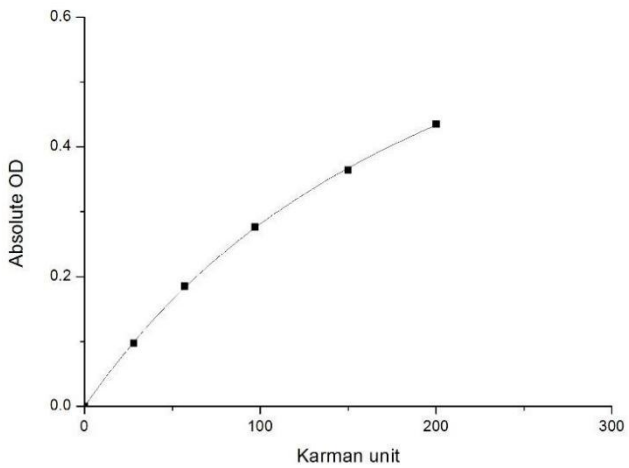
1. The kit uses Reitman-Frankel as the method, the detection result adopt Karman unit which is more accurate.

   Definition of Karman unit: 1 mL of sample, the total volume of reaction is 3 mL, wavelength is 340nm, optical path is 1 cm, react at 25℃ for 1min, the amount of generated pyruvic acid which oxidize NADH to NAD^+ and cause absorbance decreasing 0.001 is as 1 unit.

   1 Karman unit = 0.482 IU/L, 25℃.

2. The endogenous ketonic acid is generally very low in serum sample, and the $OD_{Control}$ of serum is close to the blank well (replace the serum with double-distilled water). Therefore, there is no need to set sample control for each sample and replace with blank well. But, the control well of each sample is necessary for serum with serious lipidemia, jaundice or hematolysis. If the activity of samples is out of normal or critical value, it is recommended to test again with setting control tube for each sample.
3. Dilute the serum with normal saline and re-detect if the enzyme activity is more than 150 unit.
4. Take the control well (or sample blank well) of normal serum as one of daily quality control indexes. The large difference could be caused by concentration of α-ketoglutaric acid, DNPH or instruments, etc.
5. ALT in serum can be stored for 2 days at room temperature (25℃), for 1 week at 0~4℃ and for 1 month at -20℃.

Standard curve



This manual must be read attentively and completely before using this product.
May you have any problems, please contact our Technical Service Center for help.
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