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Hepatic lipase (HL) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/ Microplate reader

Cat No: AK0232

Size:100T/48S

Components:

Reagent I: 110 mL×1. Storage at 4℃ .

Reagent II: 1.5 mL×1. Storage at 4℃ .

Reagent III: Powder×1. Storage at 4℃ . Before use, add 10 mL of distilled water, fully dissolve.

Reagent IV: Powder×2. Storage at -20℃ . Before use, add 1 mL of distilled water to the one, fully dissolve. The dissolved reagent can be stored at -20℃ after repacking. Avoid repeated freeze-thaw cycles;

Standard: Powder×1. Before use, 6.94 mL of **acetone** is added to prepare a 10 μmol/mL α-naphthol standard solution, which was fully dissolved before use.

Product Description:

Hepatic lipase (HL) is a lipolytic enzyme synthesized in liver parenchymal cells. It is present on the surface of the liver sinusoidal endothelial cells and the surface of the hepatocyte microvilli in the sinusoidal space, and can hydrolyze various lipoproteins. The triglycerides (TG) and phospholipids (PL) in the medium change the size and density of various lipoprotein particles. When the HL and its activity in the plasma increasing, it can lead to low density lipoprotein (LDL) levels in the plasma, increase and accelerate the occurrence and development of atherosclerosis.

HL hydrolyzes α-naphthyl acetate to produce α-naphthol, which can form a purple-red azo compound with fast blue B salt. It has a characteristic absorption peak at 595 nm, and its color depth is positively correlated with liver esterase activity within a certain range.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/ Microplate reader, water bath, balance, centrifuge, adjustable transferpettor, micro glass cuvette/ 96 well flat-bottom plate, mortar/homogenizer, ultrasonic crusher, ice and distilled water.

Procedure:

I. Enzyme extraction

1. Tissue

According to the tissue mass (g): the volume of Reagent I (mL) is 1:5~10 to extract. It is recommended to add 1 mL of Reagent I to 0.1 g of tissue, and fully homogenize on ice bath. Centrifuge at 10000g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.

2. Bacteria or cells

According to the bacteria or cells (10^4): the volume of Reagent I (mL) is 500~1000:1. It is recommended

to add 1 mL of Reagent I to 5 million of bacteria or cells. Use ultrasonication to splitting bacteria and cells (placed on ice, ultrasonic power 300W, working time 3s, interval 7s, total time 3 min). Centrifuge at 10000g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before testing.

3. Culture medium or other liquid: Detect directly.

II. Detection

1) Preheat spectrophotometer/ microplate reader for 30 minutes, adjust the wavelength to 595 nm, set zero with distilled water.

2) Preheat reagent III at 30℃ for more than 20 minutes.

3) Standard: Dilute the 10 μmol/mL standard solution to 5, 2.5, 1.25, 0.625, 0.3125, 0. 15625, 0.078μmol/mL with Reagent I.

4) Add the following reagents in 1.5 mL EP tubes:

	Contrast tube (C)	Test tube (T)	Standard tube (S)	Blank tube (B)
Sample (μL)	20	20	-	-
Standard solution (μL)	-	-	20	-
Reagent I (μL)	90	80	80	100
Reagent II (μL)	-	10	10	10
Mix and react for 10min at 30℃			-	-
Reagent III (μL)	80	80	80	80
Reagent IV (μL)	10	10	10	10
Mix thoroughly and detect the absorbance at 595 nm, record as A_C, A_T, A_S and A_B respectively. $\Delta A_T=(A_T-A_C)$, $\Delta A_S=(A_S-A_B)$. A contrast tube is required for each test tube, and the standard curve need only be tested once or twice.				

II. Calculation:

1. Standard curve

The concentration of standard solution as x-axis, ΔA_S as y-axis, obtain the equation y=kx+b. Take ΔA_T to the equation to acquire x (μmol/mL) value.

2. **Calculation**

1) Tissue protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the hydrolysis of α-naphthyl acetate to generate 1 μmol of α-naphthol every mg of protein in the reaction system per minute at 40℃ .

HL Activity (U/mg prot)=x×Vs÷（Vs×Cpr）÷T =0. 1x÷ Cpr

2) Tissue weight

Unit definition: One unit of enzyme activity is defined as the amount enzyme that catalyzes the hydrolysis of α-naphthyl acetate to generate 1 μmol of α-naphthol every gram of tissue in the reaction system per minute at 40℃ .

HL Activity (U/g weight) = x×Vs÷(W×Vs÷Ve)÷T =0.0333x÷ W

3) Liquid

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the

hydrolysis of α-naphthyl acetate to generate 1 μmol of α-naphthol every milliliter of liquid sample in the reaction system per minute at 40℃ .

HL Activity (U/mL) =x× Vs÷Vs÷T=0. 1x

4) Bacteria or cultured cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the hydrolysis of α-naphthyl acetate to generate 1 μmol of α-naphthol every 10^4 cells or bacteria in the reaction system per minute at 40℃ .

HL Activity (U/10^4 cell) =x× Ve÷ cell amount÷ T= 0. 1x÷ cell amount

Vs: Sample volume (mL), 0.02 mL;

Ve: Extract solution volume, 1 mL;

Cpr: Supernatant sample protein concentration (mg/mL);

T: Reaction time (min), 10 minutes;

W: Sample weight, g;

Cell amount: 10^4 cells as a unit.

Note:

1. If the sample is animal liver, it is recommended to dilute the sample with reagent I more than 25 times before testing, and multiply the dilution factor in the calculation formula.

2. If the sample is serum or plasma from obese animals, it is recommended to dilute the sample with reagent I more than 5 times before testing, and multiply the dilution factor in the calculation formula.

3. When ΔA is greater than 0.8, it is recommended to measure the sample after diluting it with the reagent, and multiply it by the dilution factor in the calculation formula.

Experimental example:

1. 0. 1g rat liver was taken for sample processing, and the supernatant is diluted 24 times, then the operation is carried out according to the operation steps. measured and calculated by 96 well plate: ΔA = AT-AB = 0.605-0.046 = 0.559, and the standard curve: y = 0.265x + 0.0033, calculate x = 2.097

HL activity (U/g mass) = $x \times V_S \div (W \times V_S \div V_{ST}) \div T \times 24$ = 50.328 U/g mass.

2. After the turkey serum was diluted 6 times, the operation was carried out according to the operation steps. measured and calculated by 96 well plate: ΔA = AT-AB =0.449-0.003=0.446, and the standard curve: y = 0.265x + 0.0033, calculate x = 1.671

HL activity (U/g mass) = $x \times V_S \div (W \times V_S \div V_{ST}) \div T \times 6$ = 1.003 U/g mass

Related Products :

AK0384/AK0383	Lipase(LPS) Activity Assay Kit
AK0530/AK0529	Triglyceride(TG) Content Assay Kit
AK0231/AK0230	Lipoprteinlipase(LPL) Activity Assay Kit
AK0297/AK0296	Plant Lipoxygenase(LOX) Activity Assay Kit
AK0536/AK0535	Free fatty Acids(FFA) Content Assay Kit