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CheKine™ Micro Hepatic Lipase (HL) Activity Assay Kit

Cat #: KTB2300 Size: 48 T/24 S 96 T/48 S

	Micro Hepatic Lipase (HL) Activity Assay Kit		
REF	**Cat #:** KTB2300	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant Tissues, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Hepatolipase (HL) is a type of lipolytic enzyme synthesized in liver parenchymal cells. It exists on the surface of endothelial cells in the perisinusoidal space of the liver and on the microvilli surface of liver cells in the perisinusoidal space. It can hydrolyze triglycerides (TG) and phospholipids (PL) in various lipoproteins, causing changes in the size and density of various lipoprotein particles. When the HL and its activity in the plasma increase, it can lead to low-density lipoprotein (LDL) in the plasma The increase of the level accelerates the occurrence and development of atherosclerosis. HL hydrolysis α-Naphthyl acetate to produce α-Naphthol, which can form a purple red azo compound with fixed blue B salt, has a characteristic absorption peak at 595 nm, and its color depth is positively correlated with HL activity within a certain range.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	70 mL	70 mL×2	4℃
Reagent Ⅱ	0.7 mL	1.4 mL	4℃, protected from light
Reagent Ⅲ	6 mL	12 mL	4℃
Reagent Ⅳ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Standard	Powder×1 vial	Powder×1 vial	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 595 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Thermostatic water bath, ice maker, centrifuge
- Deionized water, acetone
- Mortar or homogenizer

Reagent Preparation

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use; Store at 4℃.

Reagent II: Ready to use as supplied. Equilibrate to room temperature before use; Store at 4℃, protected from light.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use;If there is precipitation, it is a normal phenomenon. Let it stand at room temperature until the precipitation disappears (do not shake vigorously during this period).Store at 4℃.

Reagent IV: Prepared before use. 48 T add 0.7 mL deionized water, 96 T add 1.4 mL deionized water to fully dissolve; Unused reagents can be stored in dark at -20℃ for 2 weeks to avoid repeated freeze-thaw cycles.

Standard: Prepared before use; Add 1 mL of acetone to dissolve it into 10 μmol/mL α-Naphthol standard solution, which could be stored for 2 weeks in the dark at -20℃.

Note: Reagent II is toxic, Standard has a pungent odor, so it is recommended to experiment in a fume hood.

Standard preparation: Absorb 30 μL 10 μmol/mL α-Naphthol standard solution in EP tube, add 930 μL Reagent I to prepare 0.3125 μmol/mL α-Naphthol standard, Prepare and use immediately.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Tissue samples: Weigh 0.1 g tissue sample, add 1 mL Reagent I and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Serum (plasma) and other liquid samples: Direct determination. If the liquid is turbid, the supernatant is determined by centrifugation.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 595 nm, visible spectrophotometer was returned to zero with deionized water.
2. Preheat Reagent III at 30℃ for more than 20 min.
3. Operation table (The following operations are operated in the 96-well plate or microglass cuvette):

Reagent	Test Well (μL)	Control Well (μL)	Standard Well (μL)	Blank Well (μL)
Sample supernatant	20	20	0	0
Standard	0	0	20	0
Reagent I	80	90	80	100
Reagent II	10	0	10	10
Mix well and react at 30℃ for 10 min			No reaction required, directly add the following reagents	
Reagent III	80	80	80	80
Reagent IV	10	10	10	10

3. Mix well, let stand at 30℃ for 5 min then measure the absorbance value at 595 nm. The absorbance of test well, control well, standard well and blank well were recorded as A_{Test}, $A_{Control}$, $A_{Standard}$ and A_{Blank}. Calculate $\Delta A_{Test}=A_{Test}-A_{Control}$,

$\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Each test well needs to be equipped with a control well, standard well and blank well only need to be done once or twice. Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment. If ΔA_{Test} is less than 0.05, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.7, the sample supernatant can be further diluted by Reagent I, and the calculation result should be multiplied by the dilution multiple, or reduce the sample size for extraction.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Calculation of HL activity:

(1) Calculated by protein concentration

Unit definition: Hydrolysis per mg of protein per min α-Naphthyl acetate produces 1 μmol α-Naphthol is an enzyme activity unit.

$HL\ (U/mg\ prot)=C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Sample}\div(Cpr\times V_{Sample})\div T\times F=\mathbf{0.03125\times\Delta A_{Test}\div\Delta A_{Standard}\div Cpr\times F}$

(2) Calculated by sample fresh weight

Unit definition: Hydrolysis per g of tissue per min α-Naphthyl acetate produces 1 μmol α-Naphthol is an enzyme activity unit.

$HL\ (U/g\ fresh\ weight)=C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Sample}\div(W\times V_{Sample}\div V_{Total\ Sample})\div T\times F=\mathbf{0.03125\times\Delta A_{Test}\div\Delta A_{Standard}\div W\times F}$

(3) Calculated by sample volume

Unit definition: Hydrolysis per mL of serum per min α-Naphthyl acetate produces 1 μmol α-Naphthol is an enzyme activity unit.

$HL\ (U/mL)=C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Sample}\div V_{Sample}\div T\times F=\mathbf{0.03125\times\Delta A_{Test}\div\Delta A_{Standard}\times F}$

$C_{Standard}$: α-Naphthol standard concentration, 0.3125 μmol/mL; V_{Sample}: sample volume added, 0.02 mL; $V_{Total\ Sample}$: Reagent Ⅰ volume added, 1 mL; Cpr; sample protein concentration, mg/mL; T: reaction time, 10 min; W: sample weight, g; F: sample dilution ratio.

Typical Data

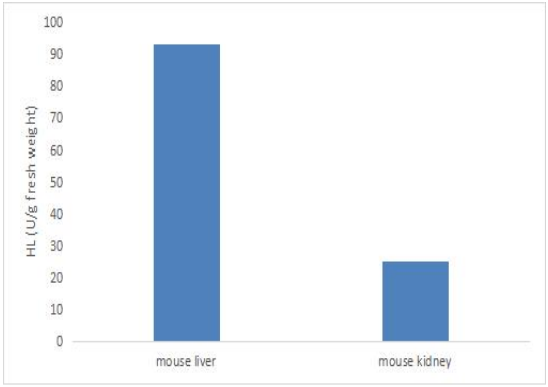

Figure 1. Determination HL activity in mouse liver and mouse kidney by this assay kit

Precautions

1. If bubbles appear in Reagent Ⅲ, it can be left to stand and wait for the bubbles to disappear. Do not inhale the bubbles when adding the sample.
2. Reagent Ⅳ may precipitate a small amount of sediment, which can be fully dissolved by blowing.
3. If the sample is animal liver, it is recommended to dilute the sample with Reagent I at least 25 times before testing, and multiply the dilution factor in the calculation formula.
4. If the sample is obese animal serum or plasma, it is recommended to dilute the sample with Reagent I at least 5 times before testing, and multiply the dilution factor in the calculation formula

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-Glucosidase Activity Assay Kit
KTB1121	CheKine™ Pyruvate Acid (PA) Colorimetric Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.