PRODUCT INFORMATION & MANUAL

Lipase Activity Assay Kit (Colorimetric)

NBP3-25861

For research use only.
Not for diagnostic or therapeutic procedures.

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Lipase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-25861

Method: Colorimetric method

Specification: 96T (Can detect 48 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.03 U/L

Detection range: 0.03-9.41 U/L

Average intra-assay CV (%): 3.0

Average inter-assay CV (%): 6.0

Average recovery rate (%: 105

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can measure lipase (LPS) activity in serum, plasma, animal tissue and cell samples.

▲Background

Lipase, also known as triglyceride hydrolase, is an enzyme with a variety of catalytic capabilities, which can catalyze the hydrolysis, alcoholysis, esterification, hydrolysis, esterification and ester reverse synthetic reaction. In addition, it also shows the activities of other enzymes, such as phospholipase, hemolysis phospholipid, cholesterol esterase, acyl peptide hydrolysis enzyme activity, etc.

▲Detection principle

Lipase can catalyze the substrate to produce sulfhydryl compounds, which react with DTNB to generate TNB. TNB has the maximum absorption at 412nm. The activity of LPS in the sample can be calculated by measuring the change of absorbance per unit time.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	60 mL × 1 vial	2-8℃ , 12 months
Reagent 2	Inhibitor	1 mL × 1 vial	2-8℃ , 12 months
Reagent 3	Substrate	5 mL × 1 vial	2-8℃ , 12 months
Reagent 4	Chromogenic Agent	1.8 mL×1 vial	2-8℃ , 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Incubator, Centrifuge, Microplate reader (400-420 nm, optimum wavelength: 412 nm)

Reagents:

Ethanol absolute (99.5%)

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. Prepare the fresh needed amount of reagent 3 working solution before use and the prepared solution should be used within 1 h.
2. It is recommended to add reagent 4 working solution under ventilation conditions due to the generated product with certain stimulating odor after incubation reaction.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 2 working solution:
   Dilute a vial of reagent 2 with ethanol absolute at the ratio of 1:9 fully. Prepare the fresh needed amount before use and the prepared solution can be stored at 2-8°C for 7 days.
3. Preparation of reagent 3 working solution:
   Mix the reagent 2 working solution and reagent 3 at the ratio of 1:100 fully. Prepare the fresh needed amount before use and the prepared solution should be used within 1 h.
4. Preparation of reagent 4 working solution:
   Dilute reagent 4 with double distilled water at the ratio of 1:9 fully. Prepare the fresh needed amount before use and the prepared solution can be stored with shading light.

▲ Sample preparation

1. Serum and plasma samples:

Detect the sample directly. If the sample is turbidity, centrifuge at 10000 g for 10 min, then take the supernatant for detection.

2. Tissue sample:

Accurately weigh the tissue, add reagent 1 at a ratio of Weight (g): Volume (ml) =1:9 and homogenize the sample in ice water bath. Then centrifuge at 10000 g for 15 min, then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant. If the supernatant is turbidity after centrifugation, repeated centrifuge until clear before use.

3. Cell sample:

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^6): reagent 1 (μL) =1: 200. Sonicate or mechanical homogenate in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.03-9.41 U/L).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor
10% Mouse liver tissue homogenate	6-10
10% Rat brain tissue homogenate	2-6
10% Rat kidney tissue homogenate	3-6
10% Rat liver tissue homogenate	5-10
10% Rat heart tissue homogenate	2-5
10% Rat lung tissue homogenate	3-6
10% Rat spleen tissue homogenate	2-5
10% Mouse lung tissue homogenate	3-6
Human serum	7-10
Human plasma	2-6

Note: The diluent is reagent 1.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'	S41	S41'
B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'	S42	S42'
C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'	S43	S43'
D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'	S44	S44'
E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'	S45	S45'
F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'	S46	S46'
G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'	S47	S47'
H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'	S48	S48'

Note: S1-S48, control wells; S1'-S48', sample wells.

▲Detailed operating steps

The measurement of samples

(1) Control well: Add 10 μL of sample to the corresponding wells.

Sample well: Add 10 μL of sample to the corresponding wells.

(2) Add 40 μL of regent 1 to control wells, add 40 μL of reagent 3 working solution to sample wells.

(3) Mix fully with microplate reader for 5 s and incubate at 37℃ for 20 min.

(4) Add 150 μL of reagent 4 working solution to each well.

(5) Mix fully with microplate reader for 5 s and incubate at 37℃ for 30 min with shading light. Measure the OD value of each well at 412 nm with microplate reader.

▲Summary operation table

	Control well	Sample well
Sample (μL)	10	10
Regent 1 (μL)	40	
Reagent 3 working solution (μL)		40
Mix fully with microplate reader for 5 s and incubate at 37℃ for 20 min.		
Reagent 4 working solution (μL)	150	150
Mix fully with microplate reader for 5 s and incubate at 37℃ for 30 min with shading light. Measure the OD value of each well at 412 nm with microplate reader.		

▲Calculation

1. Tissue and cell sample:

Definition: The amount of LPS in 1 g tissue protein per 1 minute that hydrolyze the substrate to produce 1 μmol TNB at 37°C is defined as 1 unit.

$$\text{LPS activity (U/gprot)} = \Delta A \div (\varepsilon \times b) \div C_{pr} \times f \div T \times 10^6$$

2. Serum and Plasma:

Definition: The amount of LPS in 1 L liquid sample per 1 minute that hydrolyze the substrate to produce 1 μmol TNB at 37°C is defined as 1 unit.

$$\text{LPS activity (U/L)} = \Delta A \div (\varepsilon \times b) \times f \div T \times 10^6$$

Note:

ΔA:OD_{Sample} – $OD_{Control.}$

ε: Molar absorption coefficient, 14150 $L \cdot mol^{-1} \cdot cm^{-1}$.

b: The height of the reaction system, 0.6 cm.

T: The time of incubation reaction, 20 min.

C_{pr}: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

10^6: 1 mol/L = 10^6 μmol/L.

Appendix I Data

▲Example analysis

For 10% rat liver tissue homogenate, dilute for 5 times, and carry the assay according to the operation table.

The results are as follows:

the average OD value of the control is 0.429, the average OD value of the sample is 1.513, ΔA=1.513 - 0.429 = 1.084, the concentration of protein in sample is 11.00 gprot/L, and the calculation result is:

LPS activity (U/gprot) = 1.084 ÷ (14150 × 0.6) ÷ 11.00 × 5 ÷ 20 × 10^6 = 2.90 U/gprot