PRODUCT INFORMATION & MANUAL

Lipase Activity Assay Kit (Colorimetric)

NBP3-25860

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

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Novus kits are guaranteed for 6 months from date of receipt

Lipase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-25860
Method: Colorimetric method
Specification: 50Assays **(**Can detect 48 samples without duplication)
Measuring instrument: Spectrophotometer
Sensitivity: 5.0U/L
Detection range: 5.0-2000U/L

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help.

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Application

This kit can be used to measure the lipase (LPS) content in serum, plasma and tissue samples.

Detection principle

Micelle of Emulsion formed by triglyceride and water have milky appearance because of the absorption and scattering of incident light. Triglyceride will hydrolyze under the Lipase (LPS) and the micelle will split. Thus, the rate of turbidity reduction was measured by colorimetry, and the activity of lipase was indirectly determined.

Kit components

Reagent 1: Substrate Buffer, 60 mL × 2 vials. Store at 2-8℃ for 12 months, preheat at 37℃ before use.

Reagent 2: Tris Buffer, 10 mL × 1 vial. Store at 2-8℃ for 12 months.

Reagent 3: Normal Saline, 10 mL × 1 vial. Store at 2-8℃ for a short time or -20℃ for a long-term storage.

Reagent 4: Liquid, 10 mL × 1 vial. Store sealed at 2-8℃ for 12 months.

Note: all the reagents should be brought to room temperature before detection.

Experimental instrument

Micropipettor, 37℃ Water bath, Spectrophotometer (420 nm)

Sample preparation

1. **Serum/plasma:** detect directly.
2. **20% Tissue homogenate:** Weigh the tissue sample accurately and mince the tissues to small pieces, then homogenized in normal saline on ice, the volume of normal saline (mL): the weight of the tissue (g) =4:1. The tissue homogenate is centrifuged for 10 min at 2500 rpm and collect the supernatant for detection. Meanwhile, determine the concentration of supernatant.

Detection procedures

1. For serum (plasma) sample

(1) Set the spectrophotometer at 420 nm and set to zero with reagent 2 with a 1 cm diameter cuvette.

(2) Preheat the reagent 1 at 37℃ for more than 5 min.

(3) Add 50 μL of fresh sample to the tubes, then take 2 mL of preheated reagent 1 into the tube. Mix immediately and record the time at the same time.

(4) Transfer the mixed solution to the cuvette rapidly, measure the OD value at 420 nm at 30 second (A1).

(5) Transfer the colorimetric-solution to the original tube and incubate in 37℃ water bath for 10 min accurately, and then pour the liquid to the cuvette, measure the OD value at 420 nm at 630 second (A2).

(6) **Measurement of the standard tube:** Add 50 μL of normal saline to 2 mL of reagent 1, measure the OD value at 420 nm (As). (**Note:** As value is equivalent to the absorbance of 454 umol/L standard.)

2. For tissue sample

(1) Set the spectrophotometer at 420 nm and set to zero with reagent 2 with a 1 cm diameter cuvette.

(2) Preheat the reagent 1 at 37℃ for more than 5 min.

(3) Add 25 μL of **20% tissue homogenate** supernatant to the tubes, then take 25 μL of reagent 4 and 2 mL of preheated reagent 1 into the tube. Mix immediately and record the time at the same time.

(4) Transfer the mixed solution into the cuvette rapidly, measure the OD value at 420 nm at 30 second (A1).

(5) Transfer the colorimetric-solution to the original tube and incubate in 37℃ water bath for 10 min accurately, and then pour the liquid to the cuvette, measure the OD value at 420 nm at 630 second (A2).

(6) **Measurement of the standard tube:** Add 50 μL of normal saline to 2 mL of reagent 1, measure the OD value at 420 nm (As). (Note: As value is equivalent to the absorbance of 454 umol/L standard.)

Calculation of results

1. For serum/plasma sample:

(1) **Definition:** 1 μmol of the substrate in the reaction system consumed by 1 L of serum/plasma in 1 min at 37℃ is defined as 1 LPS activity unit.

(2) **Formula:**

$$\text{LPS activity } (U/L) = \frac{A1-A2}{As} \times \text{Concentration of standard (454 } \mu mol/L) \times \frac{\text{Total volume of reaction system (2.05 } mL)}{\text{Sampling volume (0.05 } m\text{L)}} \div \text{Reaction time (10 min)}$$

2. For tissue sample:

(1) **Definition:** 1 μmol of the substrate in the reaction system consumed by 1 g tissue protein in 1 min at 37℃ is defined as 1 LPS activity unit.

(2) **Formula:**

$$\text{LPS activity } (U/gprot) = \frac{A1-A2}{As} \times \text{Concentration of standard (454 } \mu mol/L) \times \frac{\text{Total volume of reaction system (2.05 } mL)}{\text{Sampling volume (0.025 } m\text{L)}} \div \text{Reaction time (10 min)} \div \text{Protein concentration of tested sample (}gprot/L\text{)}$$

Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 12 months.
4. Do not use components from different batches of kit.
5. The cuvette must be wash with deionized water every time, and then measure the OD value.
6. The absorbance of some sample will increase after reacting with substrate. It is the samples which repeated freezing-thawing or samples with increasing IgM (such as rheumatoid factor).
7. The lipase content in pancreas is very high, so it is recommended to take a pre-experiment to determine the best sampling concentration (ΔA≤0.4, ΔA=A1-A2).