Lipase Assay Kit

Catalog No.: abx298849

Size: 50 Assays

Storage: Store all components at 4°C for short-term storage. For long-term storage (up to 6 months), store the Saline Solution at -20°C and all other components at 4°C.

Application: For quantitative detection of lipase activity in serum, plasma, and tissue homogenates.

Detection Range: 5.0 U/L – 2000 U/L

Sensitivity: 5.0 U/L

Introduction: Lipases are enzymes that perform essential roles in the digestion, transport, and processing of dietary lipids (such as fats and oils) in living organisms. In humans, pancreatic lipase is the key enzyme responsible for breaking down fats in the digestive system by converting triglycerides to monoglycerides and free fatty acids. Pancreatic lipase monitoring is also used to help diagnose Crohn's disease, cystic fibrosis, and celiac disease. Damage to the pancreas can exhibit a 5-10-fold increase of serum lipase levels within 48 hours.

Abbexa's Lipase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating lipase activity. The concentration of the reaction products of the assay can be determined by reading the absorbance of 420 nm, from which the lipase activity can be calculated.

Kit Components

1. Assay Buffer: 10 ml
2. Substrate Solution: 2 × 60 ml
3. Saline Solution: 10 ml
4. Tris Buffer: 10 ml

Materials Required But Not Provided

1. Spectrophotometer (420 nm) and cuvette
2. Centrifuge and microcentrifuge tubes
3. Water bath (set to 37°C)
4. High-precision pipette and sterile pipette tips
5. Mechanical homogenizer
6. Distilled water
7. Timer
8. Ice

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate Solution**
  Preheat to 37°C for 5 minutes immediately before use.

2. Sample

- **Serum and plasma samples**
  Serum and plasma samples can be used directly.

- **Tissue samples**
  Weigh the tissue sample accurately. For every 1 g of sample, add 0.25 ml of Saline Solution, and homogenize mechanically on ice. Transfer the sample into a centrifuge tube, and centrifuge at 2500 RPM at 4°C for 10 minutes. Transfer the supernatant (20% tissue homogenate) to a new pre-cooled tube, keep on ice and analyze immediately. The protein concentration of the supernatant should be determined separately.

B. Assay Procedure

If the expected activity and/or sample lipase concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured activity within the detection range of the kit.

Spectrophotometer cuvettes should be washed with distilled water before use.

1. Set sample, standard, and blank microcentrifuge tubes and record their positions. We recommend setting up the standard and each sample in duplicate.
2. Set the spectrophotometer to 420 nm and zero the instrument using Tris Buffer.
3. To each standard tube, add 50 µl of Saline Solution and add 2 ml of Substrate Solution (preheated). Mix thoroughly, then measure the absorbance at 420 nm. Record the OD value as $\mathbf{A_{Standard}}$, which is the equivalent to the absorbance of 454 µmol/L standard.
4. **a) Serum and plasma samples:** Add 50 µl of serum or plasma sample to each sample tube.
   b) Tissue samples: Add 25 µl of prepared 20% tissue homogenate to each sample tube, followed by 25 µl of Assay Buffer.
5. For each sample tube prepared in Step 4, add 2 ml of Substrate Solution (preheated). Mix thoroughly and start the timer.
6. Immediately transfer each solution into cuvettes and measure the absorbance at 420 nm when the timer reaches 30 seconds. Record the OD value as $\mathbf{A_1}$.
7. Transfer the solutions in the cuvettes back into the original tubes and incubate at 37°C in a water bath for 10 minutes.
8. Immediately transfer each solution into cuvettes and measure the absorbance at 420 nm when the timer reaches 10 minutes 30 seconds. Record the OD value as $\mathbf{A_2}$.
9. Calculate $\Delta\mathbf{A} = \mathbf{A_1} - \mathbf{A_2}$. It is recommended to carry out preliminary experiments and optimize the sample concentration to obtain $\Delta\mathbf{A} \leq \mathbf{0.4}$. Some samples, such as repeated freeze-thaw samples and samples with high IgM (e.g. rheumatoid factor), may cause the absorbance to increase after addition of the Substrate Solution.

C. Calculations

Lipase activity per volume of sample (in ml):

One unit of lipase activity is defined as the amount of enzyme in 1 L of solution required to consume 1 µmol of substrate per minute.

$$\mathbf{Lipase\ (U/L)} = \frac{\mathbf{C_{Standard}}}{\mathbf{T}} \times \frac{\mathbf{\Delta A}}{\mathbf{A_{Standard}}} \times \frac{\mathbf{V_{Total}}}{\mathbf{V_{Sample-Liquid}}} = \mathbf{1861.4} \times \frac{\mathbf{\Delta A}}{\mathbf{A_{Standard}}}$$

Lipase activity per mass of protein (in mg):

One unit of lipase activity is defined as the amount of enzyme in 1 g of tissue required to consume 1 µmol of substrate per minute.

$$\mathbf{Lipase\ (U/g\ protein)} = \frac{\mathbf{C_{Standard}}}{\mathbf{C_{Protein}} \times \mathbf{T}} \times \frac{\mathbf{\Delta A}}{\mathbf{A_{Standard}}} \times \frac{\mathbf{V_{Total}}}{\mathbf{V_{Sample-Tissue}}} = \frac{\mathbf{3722.8}}{\mathbf{C_{Protein}}} \times \frac{\mathbf{\Delta A}}{\mathbf{A_{Standard}}}$$

where:

$\mathbf{C_{Protein}}$	Concentration of protein (in g/L)
$\mathbf{C_{Standard}}$	Concentration of standard (454 µmol/L)
$\mathbf{T}$	Reaction time (10 minutes)
$\mathbf{V_{Total}}$	Total volume of reaction system (2.05 ml)
$\mathbf{V_{Sample-Liquid}}$	Volume of serum or plasma sample (0.05 ml)
$\mathbf{V_{Sample-Tissue}}$	Volume of tissue sample (0.025 ml)