Lipase Assay Kit

Catalog No.: abx295072

Size: 96 tests

Detection Range: 0.03 U/L – 9.41 U/L

Sensitivity: 0.03 U/L

Storage: Store all components at 4°C. Store the Chromogenic Reagent in the dark.

Application: For detection and quantification of Lipase activity in serum, plasma, tissue homogenates, and cells.

Introduction

Abbexa's Lipase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Lipase activity. Lipase catalyzes the hydrolysis of the substrate to produce sulfhydryl compounds, which react with DTNB to produce TNB, which has an absorbance maxima at 412 nm. The intensity of the color is proportional to the Lipase activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Buffer Solution: 60 ml
3. Inhibitor: 1 ml
4. Substrate: 5 ml
5. Chromogenic Reagent: 1.8 ml
6. Plate Sealer: 2

Materials required but not provided

1. Microplate reader (412 nm)
2. Double-distilled water
3. PBS (0.01 M, pH 7.4)
4. Absolute ethanol (≥ 99.5%)
5. Pipette and pipette tips
6. 1.5 ml microcentrifuge tubes
7. Centrifuge
8. Vortex mixer
9. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum and Plasma:** Serum and plasma samples can be tested directly. If any turbidity is observed, centrifuge at 10,000 × g for 10 minutes, then carefully collect the supernatant for detection. Assay immediately or store at -80°C for up to 1 month.
- **Tissue Homogenates:** Carefully weigh at least 20 mg of tissue, and wash in cold PBS (0.01 M, pH 7.4). For each 20 mg of tissue, add 180 µl of Buffer Solution. Homogenize manually using a mechanical homogenizer. Collect the tissue homogenate, then centrifuge at 10,000 × g at 4°C for 10 minutes. Carefully remove the supernatant, keep on ice, and assay immediately. Separately determine the protein concentration in the supernatant (abx097193 BCA Protein Assay Kit is recommended).
- **Adherent and Suspension Cells:** Collect 10^6 cells, and wash in PBS (0.01 M, pH 7.4). Add 200 µl of Buffer Solution, then homogenize by ultrasonication at 4°C. Centrifuge at 10,000 × g at 4°C for 10 minutes. Take the supernatant, keep on ice, and assay immediately. Separately determine the protein concentration in the supernatant (abx097193 BCA Protein Assay Kit is recommended).

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Buffer Solution, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human serum	7 – 10
Human plasma	2 – 6
10% mouse liver tissue homogenate	6 – 10
10% mouse lung tissue homogenate	3 – 6
10% rat liver tissue homogenate	5 –10
10% rat lung tissue homogenate	3 – 6
10% rat brain tissue homogenate	2 – 6
10% rat kidney tissue homogenate	3 – 6
10% rat heart tissue homogenate	2 – 5
10% rat spleen tissue homogenate	2 – 5

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Inhibitor Working Solution**: Dilute the Inhibitor with absolute ethanol at a ratio of 1:9 to prepare the Inhibitor Working Solution (e.g. add 5 µl of Inhibitor to 45 µl of absolute ethanol to prepare 50 µl of Inhibitor Working Solution). Prepare immediately before use in the assay. Store any unused Inhibitor Working Solution at 4°C for up to 7 days.
- **Substrate Working Solution**: Determine the number of wells to be used in the assay (40 µl of Substrate Reagent Working Solution is required per well). Dilute the Inhibitor Working Solution with Substrate at a ratio of 1:100 to prepare the Substrate Working Solution (e.g. add 5 µl of Inhibitor Working Solution to 500 µl of Substrate to prepare 505 µl of Substrate Working Solution). Prepare immediately before use in the assay. Unused Substrate Working Solution should be discarded if not used within 1 hour of preparation.
- **Chromogenic Reagent Working Solution**: Determine the number of wells to be used in the assay (150 µl of Chromogenic Reagent Working Solution is required per well). Dilute the Chromogenic Reagent with double-distilled water at a ratio of 1:9 to prepare the Chromogenic Reagent Working Solution (e.g. add 16 µl of Chromogenic Reagent to 144 µl of double distilled water to prepare 160 µl of Chromogenic Reagent Working Solution). Prepare immediately before use in the assay. Avoid exposure to light.

B. Assay Procedure

Allow all reagents to equilibrate to room temperature before use.

1. Set sample and control wells. Each sample requires a corresponding control. *It is strongly recommended to test samples in duplicate.*
2. Add 10 µl of sample to the sample wells and control wells.
3. Add 40 µl of Buffer Solution to the control wells.
4. Add 40 µl of Substrate Working Solution to the sample wells.
5. Tap the plate gently for 5 seconds to mix fully. Incubate at 37°C for 20 minutes.
6. Add 150 µl of Chromogenic Working Solution to each well.
7. Tap the plate gently for 5 seconds to mix fully. Incubate at 37°C for 30 minutes in the dark.
8. Measure the OD of each well with a microplate reader at 412 nm.

C. Calculation of Results

1. Serum and Plasma samples:

One unit of Lipase activity is defined as the amount of enzyme required in 1 L of sample to produce 1 µmol of TNB at 37°C.

$$\textbf{Lipase (U/L)} = \mathbf{f} \times \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Control}})}{\boldsymbol{\varepsilon} \times \mathbf{b} \times \mathbf{t}} \times \mathbf{10^6}$$

2. Tissue and Cell samples:

One unit of Lipase activity is defined as the amount of enzyme required in 1 g of tissue or cell protein to produce 1 µmol of TNB at 37°C.

$$\textbf{Lipase (U/mg protein)} = \mathbf{f} \times \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Control}})}{\boldsymbol{\varepsilon} \times \mathbf{b} \times \mathbf{t} \times \mathbf{C_{Protein}}} \times \mathbf{10^6}$$

where:

OD_{Sample}	OD value of sample
$OD_{Control}$	OD value of control
ε	Molar absorption coefficient (14150 L /mol /cm)
10^6	factor to convert mol/L to µmol/L
b	path length (0.6 cm)
$C_{Protein}$	Concentration of protein in sample (g protein/L)
t	Time of the enzymatic reaction (20 mins)
f	The dilution factor of sample prior to carrying out the assay

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.