



Lipase Activity Assay Kit (Colorimetric)

Lipase Activity Assay Kit (Colorimetric) can be used to measure Lipase activity in Serum, plasma, saliva and urine.

Catalog number: ARG82176

Package: 100 tests

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

A lipase is any enzyme that catalyzes the hydrolysis of fats (lipids). Lipases are a subclass of the esterases.

Lipases perform essential roles in digestion, transport and processing of dietary lipids (e.g. triglycerides, fats, oils) in most, if not all, living organisms. Genes encoding lipases are even present in certain viruses.

Most lipases act at a specific position on the glycerol backbone of a lipid substrate (A1, A2 or A3) (small intestine). For example, human pancreatic lipase (HPL), which is the main enzyme that breaks down dietary fats in the human digestive system, converts triglyceride substrates found in ingested oils to monoglycerides and two fatty acids.

Several other types of lipase activities exist in nature, such as phospholipases and sphingomyelinases; however, these are usually treated separately from "conventional" lipases.

Some lipases are expressed and secreted by pathogenic organisms during an infection. In particular, *Candida albicans* has many different lipases, possibly reflecting broad-lipolytic activity, which may contribute to the persistence and virulence of *C. albicans* in human tissue. [Provide by Wikipedia: Lipase]

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PRINCIPLE OF THE ASSAY

This Lipase Activity Assay Kit (Colorimetric) is a simple colorimetric assay that measures the activity of lipase in serum, plasma, saliva, urine and other biological samples. This assay is based on an improved dimercaptopropanol tributyrate (BALB) method, in which SH groups formed from lipase cleavage of BALB react with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) to form a yellow colored product. The color intensity, measured at O.D. 412 nm, is proportionate to the enzyme activity in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped at room temperature. Store all components at 4°C upon receiving. Shelf life: 12 months after receipt.

Component	Quantity	Storage information
Assay Buffer (pH 8.5)	15 mL	4°C
Color Reagent (530 mg)	1 vial	4°C
BALB Reagent	1 mL	4°C
Standard (equivalent to 735 U/L)	2 mL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 570 nm
- Clear flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Lipase inhibitors (EDTA, and certain detergents Tween-20, NP-40), β -mercaptoethanol and dithiothreitol interfere with this assay and should be avoided in sample preparation.
- This assay is based on a kinetic reaction, addition of the Working Reagent should be quick. Use of a multi-channel pipettor is recommended.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Collect the serum and assay directly.

Tissue and cell lysate: homogenization in 1X cold PBS and centrifugation for 5 minutes at 14,000 rpm.

Other liquid biological sample: assay directly.

Note:

- Lipase inhibitors (EDTA, and certain detergents Tween-20, NP-40), β -mercaptoethanol and dithiothreitol interfere with this assay and should be avoided in sample preparation.
- Samples can be stored frozen for at least one month, if not assayed immediately.

REAGENT PREPARATION

- **Working Reagent:** for each reaction, mix 5 mg of Color Reagent, 140 μL of Assay Buffer and 8 μL of BALB Reagent. The Working Reagent should be prepared freshly and used within one hour.

ASSAY PROCEDURE

Equilibrate reagents to room temperature. Briefly centrifuge tubes before use.

	Standard wells	Sample wells	Control wells
Standards	150 μL		
Distilled water			150 μL
Samples		10 μL	
Working Reagent		140 μL	
Tap plate to mix briefly. Incubate for 10 minutes and 20 minutes at room temperature or 37°C .			
Read the absorbance at O.D. 412 nm at 10 minutes (OD_{10min}) and 20 minutes (OD_{20min}) .			

Note: if the assay is to be performed at another temperature (E.g., 37°C), warm up the Working Reagent to this temperature prior to adding to the sample.

CALCULATION OF RESULTS

1. Lipase activity is calculated as follows:

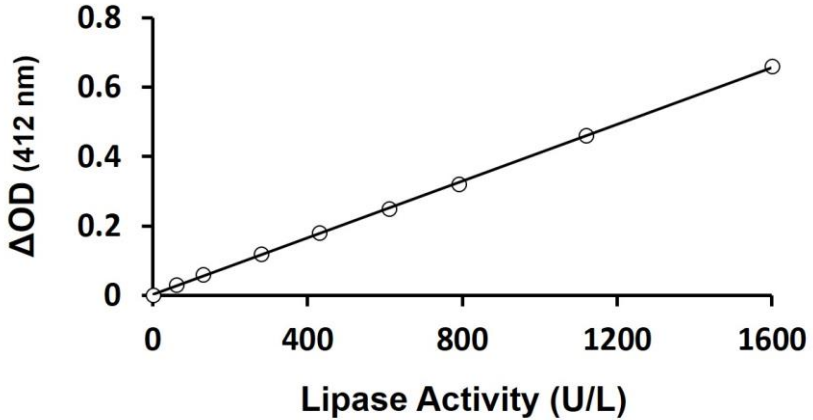
$$\text{Lipase Activity (U/L)} = [(OD_{20\text{min}} - OD_{10\text{min}}) / (OD_{\text{Standard}} - OD_{\text{H}_2\text{O}})] \times 735$$

Note:

- $OD_{20\text{min}}$, $OD_{10\text{min}}$: the O.D. 412 nm values of the sample at 20 minutes and 10 minutes.
 - OD_{Standard} , $OD_{\text{H}_2\text{O}}$: the O.D. 412 nm values of the Standard and distilled water at 20 minutes.
 - 735: equivalent activity (U/L) of the Standard under assay conditions.
2. Unit definition: one unit of enzyme catalyzes the cleavage of 1 μmole of substrate per minute under the assay conditions (pH 8.5).
 3. If the calculated activity is higher than 1600 U/L, dilute sample in water and repeat assay. Multiply the results by the dilution factor (n).

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Lipase Activity Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

40 U/L