Technical Bulletin

Lipase Assay Kit

Catalogue Number MAK482

Product Description

Lipase catalyzes the hydrolysis of ester bonds on the glycerol backbone of a lipid substrate. 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. Human pancreatic lipase and its related protein 2 are the main lipases secreted by the pancreas. In acute pancreatitis, lipase levels can rise 5 to 10-fold within 24 to 48 hours. Increased activities have also been associated with pancreatic duct obstruction, pancreatic cancer, kidney disease, salivary gland inflammation, bowel obstruction, and other pancreatic diseases. Decreased levels may indicate permanent damage to lipase-producing cells in the pancreas.

Simple, direct and automation-ready procedures for measuring lipase activity are useful. The Lipase Assay Kit is based on an improved Dimercaptopropanol tributyrate (BALB) method, in which thiol (SH) groups formed from lipase cleavage of BALB react with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) to form a colored product. The color intensity, measured at 412 nm, is proportionate to the enzyme activity in the sample.

The linear detection range is 40 to 1600 U/L lipase activity in a 96-well plate assay. The kit is suitable for detecting lipase in serum, plasma, saliva, urine and other biological samples.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Assay Buffer (pH 8.5) 15 mL
  Catalogue Number MAK482A
- Color Reagent 530 mg
  Catalogue Number MAK482B
- BALB Reagent 1 mL
  Catalogue Number MAK482C
- Calibrator (equivalent to 735 U/L) 2 mL
  Catalogue Number MAK482D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (such as, multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL microcentrifuge tubes
- Dounce tissue grinder set
  (Catalogue Number D9063 or equivalent)
- Microcentrifuge capable of RCF ≥ 14,000 × *g*
- Phosphate Buffered Saline (PBS)
  (Catalogue Number P3813 or equivalent)

For Cuvette Method Only

- 1 mL cuvettes
- Spectrophotometer

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped in room temperature. Store components at 2-8 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Lipase inhibitors (EDTA and certain detergents, such as 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.

Tissues and Cells

Homogenize tissue (10 mg) or cells (10^6) in 200 µL of cold 1× PBS and then centrifuge for 5 minutes at room temperature at 14,000 × *g* to pellet any debris. Use the clear supernatant for the assay.

Procedure Using 96-Well Plate

All Samples

Transfer 10 µL of each Sample into separate wells of a 96-well plate.

Blank

Transfer 150 µL of purified water into separate wells of a 96-well plate.

Calibrator

Transfer 150 µL of Calibrator into separate wells of a 96-well plate.

Working Reagents

This assay is based on an enzyme-catalyzed kinetic reaction. To ensure identical incubation time, addition of Working Reagent should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

1. Mix enough reagents for the number of assays to be performed. For each Sample well, prepare Working Reagent according to Table 2. The Working Reagent should be prepared fresh and used within one hour of preparation.

   If the assay is to be performed at another temperature (such as 37 °C), warm the Working Reagent to this temperature prior to adding to the Sample.

Table 1.
Preparation of Working Reagent

Reagent	Working Reagent
Assay Buffer	140 µL
Color Reagent	5 mg
BALB Reagent	8 µL

2. Add 140 µL of Working Reagent to each **Sample** well. Do not add Working Reagent to Blank or Calibrator wells.
3. Tap plate briefly to mix reaction mixture.

Measurement

1. Incubate the plate for 10 minutes at room temperature.
2. Read optical density at 412 nm for time (OD_{10}).
3. Incubate the plate for an additional 10 minutes at room temperature.
4. At the 20-minute incubation point, read the plate again at 412 nm (OD_{20}). Alternatively, monitor the optical density of the plate for 20 minutes in kinetic mode at room temperature at 412 nm and record the 10-minute and 20-minute OD readings.

Procedure using 1 mL cuvette

1. Transfer 1 mL of purified water for Blank to a cuvette.
2. Transfer 1 mL of Calibrator to a cuvette.
3. Transfer 60 µL of each Sample to separate cuvettes.
4. Add 940 µL of Working Reagent to each **Sample** cuvette. Do not add Working Reagent to Blank or Calibrator cuvettes.
5. Incubate the cuvettes for 10 minutes at room temperature.
6. Read optical density at 412 nm for time (OD_{10}).
7. Incubate the cuvettes for an additional 10 minutes at room temperature.
8. At the 20-minute incubation point, read the cuvettes again at 412 nm (OD_{20}). Alternatively, monitor the optical density of the cuvettes for 20 minutes in kinetic mode at room temperature at 412 nm and record the 10-minute and 20-minute OD readings.

Results

Calculate the lipase activity of the Sample using the below formula.

Lipase activity (U/L) =

$$\frac{OD_{20} - OD_{10}}{OD_{Calibrator} - OD_{Blank}} \times 735$$

where:

OD_{20} = Optical density reading of Sample at 20 minutes

OD_{10} = Optical density reading of Sample at 10 minutes

$OD_{calibrator}$ = Optical density reading of Calibrator at 20 minutes

OD_{Blank} = Optical density reading of Blank at 20 minutes

735 = Equivalent activity (U/L) of the Calibrator under the assay conditions

If the calculated activity is higher than 1600 U/L, dilute Sample in purified water and repeat assay. Multiply the results by the dilution factor (DF).

Unit definition: One unit of enzyme catalyzes the cleavage of 1 µmole of substrate per minute under the assay conditions (pH 8.5).

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