

## User's Manual and Instructions

### Lipase Assay Kit (Z5030051)

Rapid Colorimetric Determination of Lipase Activity at 412nm

#### 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 very desirable. BioChain's Lipase Assay is based on an improved dimercaptopropanol tributyrates (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 412 nm, is proportionate to the enzyme activity in the sample.

#### APPLICATIONS

Direct assays of lipase activity in serum, plasma, saliva, urine and other biological samples.

#### KEY FEATURES

**Sensitive and accurate.** Linear detection range 40 to 1600 U/L lipase activity in 96-well plate assay.

**Convenient and high throughput.** The procedure involves adding a single working reagent, and reading the optical density at 10 min and 20 min at room temperature or 37°C. Can be automated to process thousands of samples per day.

#### KIT CONTENTS (100 tests in 96-well plate)

**Assay Buffer (pH 8.5): 15 mL**

**Color Reagent: 530 mg      BALB Reagent: 1.0 mL**

**Calibrator: 2.0 mL (equivalent to 735 U/L)**

**Storage conditions.** This product is shipped at room temperature. Store all components at 4°C. Shelf life: 12 months after receipt.

**Precautions:** 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.

#### PROCEDURES

**Sample Preparation.** Lipase inhibitors (EDTA, and certain detergents Tween-20, NP-40), 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. Tissue and cell lysates can be obtained by homogenization in cold PBS buffer and centrifugation (e.g. 5 min at 14,000 rpm).

**Preparation of Working Reagent.** Mix Color Reagent into Assay Buffer and shake vial to mix. Add 0.8 mL BALB Reagent (sufficient for 100 assays). Alternatively for partial reconstitution: for each well of reaction, mix 5 mg Color Reagent, 140 µL Assay Buffer and 8 µL BALB Reagent. *The Working Reagent should be prepared freshly and used within one hour.*

**Important:** this assay is based on a kinetic reaction, addition of the Working Reagent should be quick. Use of a multi-channel pipettor is recommended.

1. Transfer 150 µL H<sub>2</sub>O and 150 µL Calibrator into wells of a clear-bottom 96-well plate. Pipette 10 µL samples into separate wells. Add 140 µL Working Reagent to each sample well. Tap plate briefly to mix reaction mixture. *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.*

2. Read OD<sub>412nm</sub> on a plate reader at 10 min (OD<sub>10min</sub>) and at 20 min (OD<sub>20min</sub>).
3. **Calculation:** lipase activity is calculated as follows,

$$\text{Activity} = \frac{\text{OD}_{20\text{min}} - \text{OD}_{10\text{min}}}{\text{OD}_{\text{Calibrator}} - \text{OD}_{\text{H}_2\text{O}}} \times 735 \quad (\text{U/L})$$

where OD<sub>20min</sub> and OD<sub>10min</sub> are the OD<sub>412nm</sub> values of the sample at 20 min and 10 min, respectively. OD<sub>Calibrator</sub> and OD<sub>H<sub>2</sub>O</sub> are the OD<sub>412nm</sub> values of the Calibrator and water at 20 min. The number "735" is the equivalent activity (U/L) of the calibrator under the assay conditions.

**Note:** 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*).

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

#### MATERIALS REQUIRED, BUT NOT PROVIDED

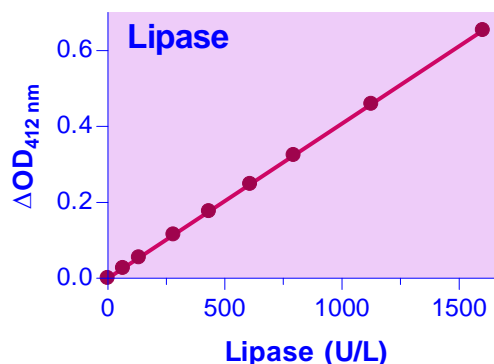
Pipeting (multi-channel) devices. Clear-bottom 96-well plates (e.g. Corning Costar) and plate reader.

#### FOR ASSAYS IN CUVETTE

For assays in standard 1 mL cuvet, use 1 mL H<sub>2</sub>O and 1 mL Calibrator. Reaction volumes: 60 µL sample + 940 µL Working Reagent.

#### EXAMPLES

Samples were assayed in duplicate using the 96-well plate protocol. Lipase activities were 809 ± 15 U/L for mouse serum, 959 ± 23 U/L for rat plasma, 665 ± 14 U/L for rat serum, 44 ± 1 U/L for goat serum, 80 ± 1 U/L for bovine serum, 75 ± 6 U/L for human serum and 52 ± 2 U/L for a human plasma sample.



#### LITERATURE

1. Furukawa, I. et al (1982). Assays of serum lipase by the "BALB-DTNB method" mechanized for use with discrete and continuous-flow analyzers. *Clin Chem.* 28: 110-113.
2. Lombard, S. et al (2001). Lipolytic activity of ricin from *Ricinus sanguineus* and *Ricinus communis* on neutral lipids. *Biochem J.* 358: 773-781.
3. Kurooka S. and Kitamura T. (1978). Properties of serum lipase in patients with various pancreatic diseases. Analysis by a new serum lipase assay method (the BALB-DTNB method) in combination with gel-filtration and iso-electrofocusing techniques. *J Biochem (Tokyo).* 84:1459-66.