For research use only

Lipase Activity Assay Kit II

(Catalog #K723-100; 100 assays; Store kit at -20°C)

I. Introduction:

Lipase is a subclass of the esterases that catalyze the hydrolysis of ester bonds in water-insoluble, lipid substrates. Lipases perform essential roles in the digestion, transport and processing of dietary lipids (e.g. triglycerides, fats, oils) in most, if not all, living organisms. In humans, pancreatic lipases are the key enzyme responsible for breaking down fats in the digestive system by converting triglycerides to monoglycerides and free fatty acids. During the damage of the pancreas, lipase levels can rise 5 to 10-fold within 24 to 48 hours. The kit provides a simple, sensitive, and reliable assay for rapid analysis of Lipase in samples. In the assay, lipases hydrolyze a specific substrate to generate a product which reacts with the DTNB probe to generate color (λ = 412 nm). The kit is also suitable for high throughput analyses.

II. Kit Contents:

Components	100 assays	Cap Code	Part Number
Lipase Assay Buffer	25ml	WM	K723-100-1
DTNB Probe (lyophilized)	1 vial	Red	K723-100-2
Lipase Substrate	0.5 ml	Blue	K723-100-3
TNB Standard (2.5 µmol; lyophilized)	1 vial	Amber	K723-100-4
Lipase Positive Control (lyophilized)	1 vial	Purple	K723-100-5

III. Storage and Handling:

Store the kit at -20°C, protect from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol prior to performing the assay.

IV. Reagent Preparation:

DTNB Probe: Dissolve the DTNB Probe with 1.1 ml Lipase Assay Buffer. Store at -20°C. Use within two months.

Lipase Substrate: Ready to use. Store at -20°C. Use within two months.

TNB Standard: Dissolve in 0.5 ml of Lipase Assay Buffer to generate a 5 mM TNB Standard. Aliquot and store at -20 °C; avoid freeze-thaw cycles. The TNB standard solution is stable for 1 week at 4°C and 1 month at -20°C.

Lipase Positive Control: Dissolve the positive control with 100 µl Lipase Assay Buffer. Store at -20°C. Use within two months.

V. Lipase Assay Protocol:

1. **Standard Curve Preparation:**
   Add 0, 2, 4, 6, 8, 10 µl of TNB Standard into a series of wells. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 10, 20, 30, 40, 50 nmol/well of TNB Standard.
2. **Sample Preparations:**
   Tissues or cells can be homogenized in 4 volumes of Assay Buffer and centrifuged (13,000 x g, 10 min) toremove insoluble material. Serum samples can be directly diluted in the Assay Buffer. Prepare test samples of up to 50 µl/well with Assay Buffer in a 96-well plate. We suggest testing several doses of your sample to make sure the readings are within the standard curve range. Mercaptans in samples will cause a high background. If the sample background is too high, the sample can be precipitated with 2 volumes of saturated ammonia sulfate. Then centrifuge, collect the precipitates and re-dissolve in the same volume of assay buffer to remove small molecule mercaptans.
3. **Positive Control (optional):**
   Add 5 µl of the reconstituted Lipase Positive Control into Positive Control well and adjust the volume to 50 µl/well with assay buffer.
4. **Reaction Mix:** Mix enough reagent for the number of assays to be performed. For each well, prepare a total 100 µl Reaction Mix:

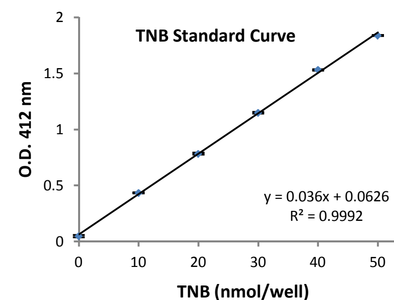
   85 µl Assay Buffer
   10 µl DTNB Probe
   5 µl Lipase substrate

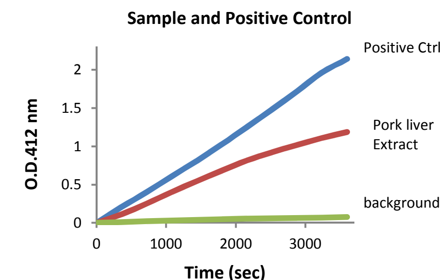
   Add 100 µl of the Reaction Mix to each well containing the Positive Controls and samples. Mix well. **(DO NOT ADD TO STANDARDS)**
5. **Measurement:** Read OD 412 nm A_1 at T_1 after 3 min incubation time. Read A_2 OD 412 nm again at T_2 after incubating the reaction at 37°C for 60 - 90 min (or incubate longer time if the Lipase activity is low), protect from light. The OD of color generated upon formation of TNB is ΔA 412 nm = $A_2 - A_1$. It is recommended to read kinetically to choose the A_1 and A_2 values which are in the linear range of the Standard Curve.
6. **Calculation:** Subtract 0 Standard from all standard readings. Plot theStandard Curve. Apply the ΔA 412 nm of samples to the standard curve to get B nmol of TNB generated in the sample reaction between T_1 and T_2. Lipase activity in samples can then be calculated:

$$\text{Lipase Activity} = \frac{B}{(T2-T1)\times V} \times \text{Sample Dilution Factor} = \text{nmol/min/ml} = \text{mU/ml}$$

Where: **B** is the TNB amount calculated from theStandard Curve (in nmol).
T_1 is the time of the first reading (A_1) (in min).
T_2 is the time of the second reading (A_2) (in min).
V is the pretreated sample volume added into the reaction well (in ml).

Unit Definition: One unit lipase is defined as the amount of lipase which hydrolyzes the substrate and generates 1.0 µmol of TNB per minute at 37°C.





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Fatty Acid Assay Kit
Uric Acid Assay Kit
Lactate Assay Kit/ II
Creatinine Assay Kit
Free Glycerol Assay Kit
Triglyceride Assay Kit
Cholesterol Assay Kits

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GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed • Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		