ab102525

Lipase Detection Kit II (Colorimetric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Lipase activity in various samples.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.
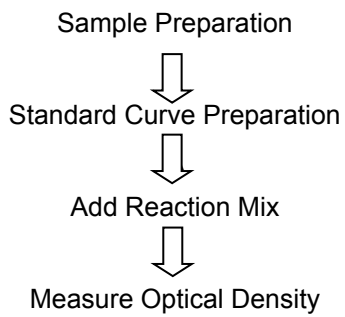
Version 5a, Last Updated 21 August 2023

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1. Overview

Lipases are 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.

Abcam's Lipase Detection Kit II (Colorimetric) 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/DTNB probe to generate color (λ=412 nm). The kit is also suitable for high throughput analyses.

2. Protocol Summary

Sample Preparation

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Standard Curve Preparation

⇩

Add Reaction Mix

⇩

Measure Optical Density

3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer V/Assay Buffer	25 mL
DTNB/DTNB Probe (Lyophilized)	1 vial
Lipase Substrate II/Lipase Substrate	0.5 mL
TNB Standard/TNB Standard (Lyophilized)	1 vial
Lipase Positive Control/Lipase Positive Control (Lyophilized)	1 vial

Store kit at -20°C, protect from light. Warm the assay buffer to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

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

LIPASE SUBSTRATE II/LIPASE SUBSTRATE: Ready to use. Store at -20°C. Use within two months.

TNB STANDARD: Dissolve with 0.5 mL of Assay Buffer V/Assay Buffer to generate 5 mM TNB Standard. Stable for 2 months at -20°C.

LIPASE POSITIVE CONTROL: Dissolve the positive control with 100 µl Assay Buffer V/Lipase Assay Buffer. Store at -20°C. Use within two months.

B. Additional Materials Required

- Microcentrifuge

- Pipettes and pipette tips

- Colorimetric microplate reader

- 96-well plate

- Orbital shaker

4. Assay Protocol

1. Sample Preparation:

a. **For serum samples:** Serum samples can be directly diluted in the Assay Buffer V/Assay Buffer.

b. **For tissue or cell samples:** Tissues or cells can be homogenized in 4 volumes of Assay Buffer V/Assay Buffer and centrifuged (13,000 x g, 10 min) to remove insoluble material.

Prepare test samples of up to 50 µl/well with Assay Buffer V/Assay Buffer in a 96-well plate.

We suggest testing several doses of your sample to make sure readings are within the standard curve.

Note:

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.

2. 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.

3. Standard Curve Preparation:

Add 0, 2, 4, 6, 8, 10 µl of TNB Standard into a series of wells. Adjust volume to 150 µl/well with Assay Buffer V/Assay Buffer to generate 0, 10, 20, 30, 40, 50 nmol/well of TNB Standard.

4. Reaction Mix: Mix enough reagent for the number of assays to be performed. For each well, prepare a total 100 µl Reaction Mix.

Assay Buffer V/Assay Buffer	85 µl
DTNB/DTNB Probe	10 µl
Lipase Substrate II/Lipase substrate	5 µl

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_{412nm} A_1 at T_1 after 3 min incubation time. Read A_2 OD_{412nm} 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:

$$\Delta A_{412nm} = A_2 - A_1.$$

Note:

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.

5. Data Analysis

Subtract zero Standard from all standard readings. Plot the Standard Curve.

Apply the Δ_{A412nm} 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:

$$\textbf{Lipase Activity } = \frac{\textbf{(B x Dilution Factor)}}{\textbf{(T}_2 - \textbf{T}_1\textbf{) x V}} = \textbf{nmol/min/ml = mU/ml}$$
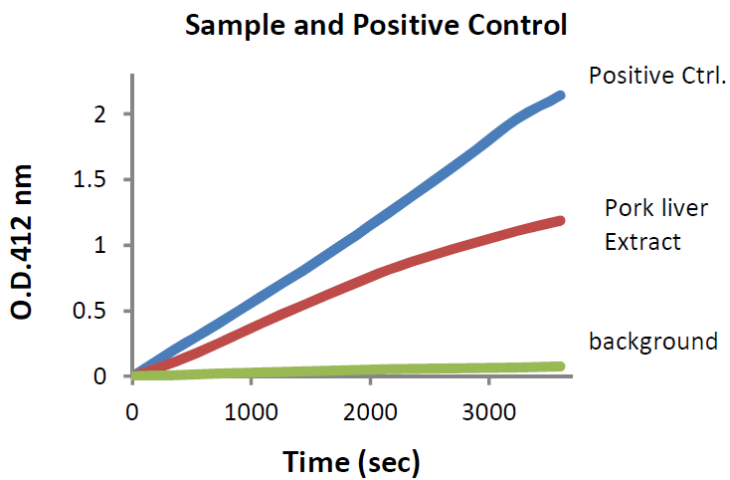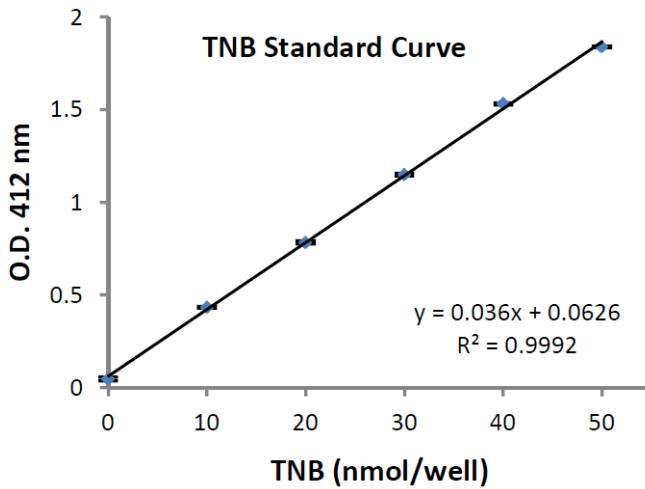
Where:

B is the TNB amount from the Standard 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 pre-treated sample volume (ml) added into the reaction well

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.

TNB Standard Curve

$y = 0.036x + 0.0626$

$R^2 = 0.9992$

Sample and Positive Control

Positive Ctrl.

Pork liver Extract

background

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the *10kDa spin column (ab93349)*
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

Technical Support

For all technical or commercial enquiries please go to: www.abcam.com/contactus

www.abcam.cn/contactus (China)

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