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# 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.

Version 6a, Last Updated 29 May 2025

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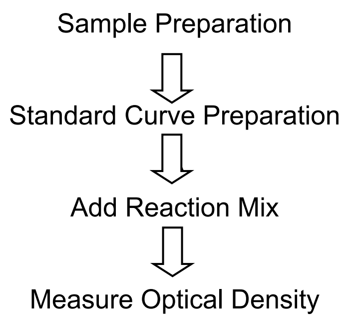
# 1. Overview

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 to generate color (λ=412 nm). The kit is also suitable for high throughput analyses.

## 2. Protocol Summary



# 3. Components and Storage

## A. Kit Components

| Item | Quantity |
|---|---|
| Assay Buffer 5 | 25 mL |
| DTNB | 1 vial |
| Lipase Substrate II | 500 µL |
| TNB Standard | 1 vial |
| Lipase Positive Control | 1 vial |

PLEASE NOTE: Assay Buffer 5 was previously labelled as Assay Buffer V and Assay Buffer. The composition has not changed.

Store kit at -20°C, protect from light. Warm the Assay Buffer 5 to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

DTNB: Dissolve the DTNB with 1.1 ml Assay Buffer 5. Store at -20°C. Use within two months.

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

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

LIPASE POSITIVE CONTROL: Dissolve the positive control with 100 µl of Assay Buffer 5. 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 5.

**b. For tissue or cell samples:** Tissues or cells can be homogenized in 4 volumes of Assay Buffer 5 and centrifuged (13,000 x g, 10 min) to remove insoluble material.

Prepare test samples of up to 50 µl/well with Assay Buffer 5 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 5 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 5 | 85 µl |
| DTNB | 10 µl |
| Lipase Substrate II | 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 $\Delta_{A412nm}$ 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:

$$\textbf{Lipase Activity} = \frac{(B \times \text{Dilution Factor})}{(T_2 - T_1) \times V} = \text{nmol/min/ml} = \text{mU/ml}$$

Where:

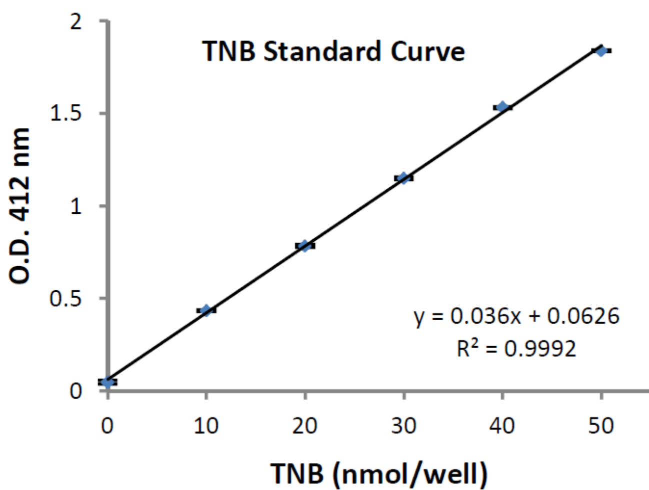
**B** is the TNB amount from the Standard Curve (in nmol).

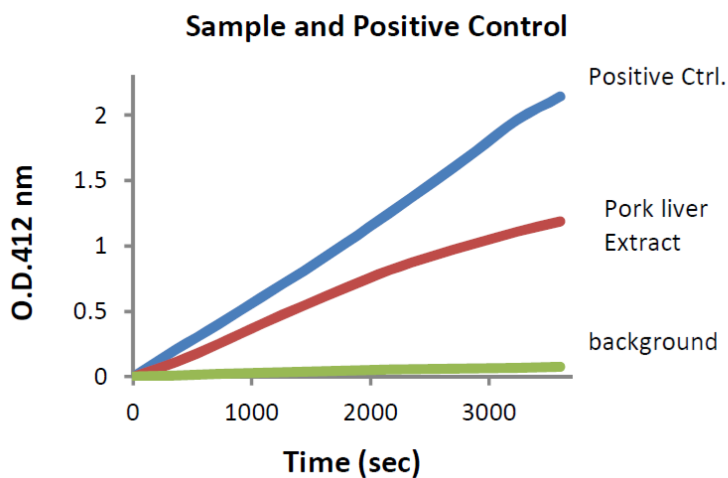
$\mathbf{T_1}$ is the time of the first reading ($A_1$) (in min).

$\mathbf{T_2}$ 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
O.D. 412 nm
TNB (nmol/well)
y = 0.036x + 0.0626
R² = 0.9992
0
0.5
1
1.5
2
0
10
20
30
40
50


Sample and Positive Control
O.D.412 nm
Time (sec)
Positive Ctrl.
Pork liver
Extract
background
0
0.5
1
1.5
2
0
1000
2000
3000

## 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);<br>Luminescence: White plates;<br>Colorimetry: Clear plates.<br>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**

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