ab118969

Lipase Detection Kit III (Fluorometric)

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 5a, Last Updated 29 May 2025

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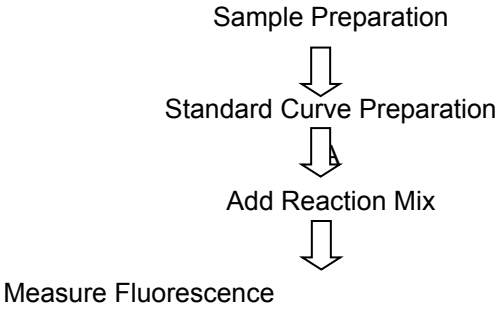
1. Overview

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.

In Abcam's Lipase Detection Kit III (Fluorometric), Lipase hydrolyzes a specific substrate to generate the methylresorufin, which can be detected fluorometrically at Ex/Em=529/600 nm. The kit provides a rapid, simple, more sensitive, and reliable test suitable for high throughput assay of Lipase activity. This kit can be used to detect Lipase as low as 0.1 µU/well.

2. Protocol Summary



3. Materials Supplied

Item	Quantity
Assay Buffer 5	25 mL
Lipase Substrate I	200 µL
Methylresorufin Standard	40 µL
Lipase Positive Control	1 vial

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

Please note the Lipase Substrate I can range in color from faint orange to orange to red.

4. Storage and Stability

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

LIPASE POSITIVE CONTROL: Reconstitute with 100 µl Assay Buffer 5. Mix 2 µl Positive Control with 998 µl Assay Buffer 5. Aliquot and store reconstituted Lipase positive control at -20°C. Use within two months.

5. Materials Required, Not Supplied

- Microcentrifuge
- Pipettes and pipette tips
- Fluorometric microplate reader
- 96-well plate
- Orbital shaker

6. Assay Protocol

1. Sample Preparation:

a. **For liquid samples (cell culture media, cell culture supernatant, milk, plasma, serum, urine and other biological fluids):** liquid samples can be assayed directly or after dilution in Assay Buffer 5. You might want to test different sample volumes to find the optimal that will give you a reading within the linear range of the standard curve.

b. **For tissue or cell samples:** Tissues (50 mg) or cells ($1x10^6$) can be homogenized in ~200 µl ice-cold Assay Buffer 5 then centrifuged to remove insoluble material at 13,000 x g, 10 min.

   Prepare test samples on up to 50 µl/well with Assay Buffer 5 in a 96-well plate.

c. **Lipase positive control:** dilute 2 µl of reconstituted Lipase positive control into 998 µl Assay Buffer 5 (Diluted Lipase control).
   Add 10 µl of the Diluted Lipase control to a microcentrifuge tube and top up to 250 µl with Assay Buffer 5 (240 µl).
   Transfer 50 µl to a well to use as positive control to the reaction (there is enough amount for duplicate samples).

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

2. Standard Curve Preparation:
Add 10 µl of the Methylresorufin Standard to 90 µl Assay Buffer 5 to generate a 10 µM standard solution.
Add 0, 2, 4, 6, 8, 10 µl to each well individually.

Adjust the volume to 100 µl/well with Assay Buffer 5 to generate 0, 20, 40, 60, 80, 100 pmol/well of Methylresorufin Standard. Read fluorometrically at Em/Ex = 529/600nm.

3. Reaction Mix: Mix enough reagents for the number of assays to be performed. Thaw Lipase Substrate I completely before use.

For each well, prepare a total 50 µl Reaction Mix.

Assay Buffer 5	48 µl
Lipase Substrate I	2 µl

Add 50 µl of the Reaction Mixes to each well containing the samples and positive controls. Mix well. Include a reagent background control by adding 50 µl assay buffer to 50 µl reaction mix into a well.

4. Measurement:

Read Ex/Em = 529/600nm R_1 for sample and R_{1B} for background control at T_1.

Read R_2 for sample and R_{2B} for background control again at T_2 after incubating the reaction at 37°C for 30-60 min (or incubate longer time if the Lipase activity is low), protect from light.

The fluorescence generated by the hydrolysis of the Lipase Substrate I is:

$$\mathbf{\Delta RFU = (R_2 - R_{2B}) - (R_1 - R_{1B}).}$$

Note:

It is recommended to read the fluorescence kinetically to choose the R_1 and R_2 within the linear range of the standard curve.

7. Data Analysis

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

Apply the ΔRFU to the standard curve to get B nmol of Methylresorufin generated between T_1 and T_2 in the reaction wells:

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

Where:

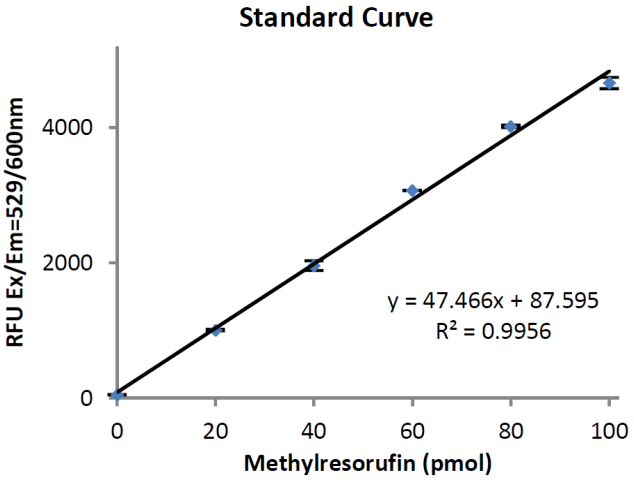
B is the Methylresorufin amount from the Standard Curve (in nmol).

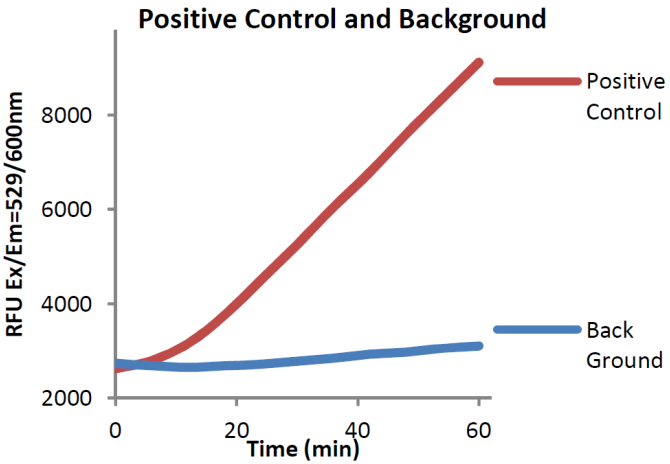
T_1 is the time of the first reading (R_1) (in min).

T_2 is the time of the second reading (R_2) (in min).

V is the pre-treated sample volume (ml) added into the reaction well

Unit Definition: One unit is defined as the amount of enzyme that hydrolyzes the substrate to yield 1.0 µmol of Methylresorufin per minute at 37°C.


Standard Curve
RFU Ex/Em=529/600nm
4000
2000
0
y = 47.466x + 87.595
R² = 0.9956
0
20
40
60
80
100
Methylresorufin (pmol)


Positive Control and Background
RFU Ex/Em=529/600nm
8000
6000
4000
2000
0
20
40
60
Time (min)
Positive Control
Back Ground

8. 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

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)