

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.

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.

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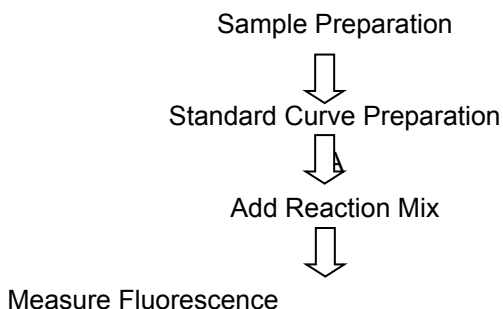
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1. Overview

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 μ U/well.

2. Protocol Summary



3. Materials Supplied

| Item | Quantity |
|---|------------|
| Assay Buffer V/Assay Buffer | 25 mL |
| Lipase Substrate I/Lipase Substrate | 0.2 mL |
| Methylresorufin Standard/Methylresorufin Standard (0.1 mM) | 40 μ L |
| Lipase Positive Control/Lipase Positive Control (Lyophilized) | 1 vial |

Please note the Lipase Substrate 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 V/Assay Buffer to room temperature before use.

Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

LIPASE POSITIVE CONTROL: Reconstitute with 100 μ l Assay Buffer V/Assay Buffer. Mix 2 μ l Positive Control with 998 μ l Assay Buffer V/Lipase Assay Buffer. 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:

- 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 V/Lipase Assay Buffer. 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.
- For tissue or cell samples:** Tissues (50 mg) or cells (1×10^6) can be homogenized in ~200 μ l ice-cold Assay Buffer V/Lipase Assay Buffer then centrifuged to remove insoluble material at 13,000 x g, 10 min.

Prepare test samples on up to 50 μ l/well with Assay Buffer V/Assay Buffer in a 96-well plate.

c. Lipase positive control: dilute 2 μl of reconstituted Lipase positive control into 998 μl Assay Buffer V/Lipase Assay Buffer (Diluted Lipase control).

Add 10 μl of the Diluted Lipase control to a microcentrifuge tube and top up to 250 μl with Assay Buffer V/Lipase Assay Buffer (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/0.1 mM Methylresorufin Standard to 90 μl Assay Buffer V/Lipase Assay Buffer 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 V/Lipase Assay Buffer 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/Lipase Substrate completely before use.

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

| | |
|-------------------------------------|------------------|
| Assay Buffer V/Assay Buffer | 48 μl |
| Lipase Substrate I/Lipase substrate | 2 μl |

Add 50 μ l of the Reaction Mixes to each well containing the samples and positive controls. Mix well. Include a reagent background control by adding 50 μ l assay buffer to 50 μ l 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 /Lipase substrate is:

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

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

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

Where:

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

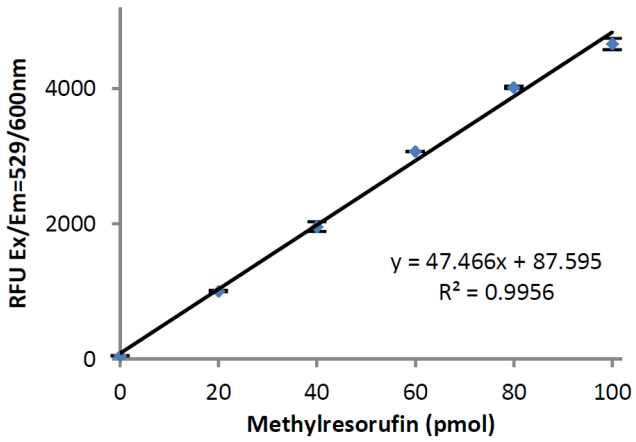
T_1 is the time of the first reading (R_1) (in min).

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

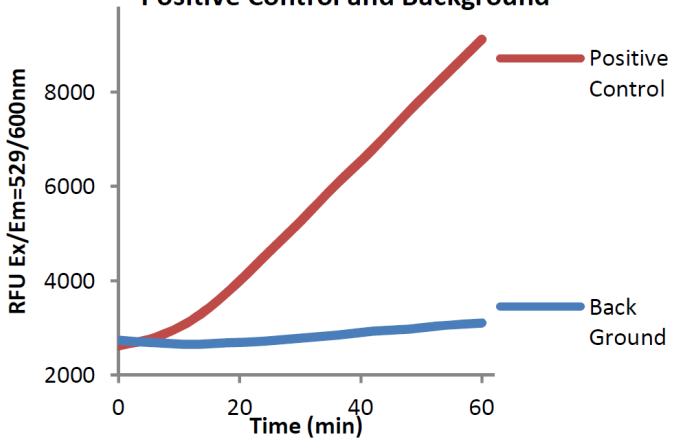
V is the pre-treated sample volume (ml) added into the reaction well

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



Positive Control and Background



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

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