

Version 11a, Last updated 15 August 2023

ab102524

Lipase Activity Assay Kit (Colorimetric)

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

[View kit datasheet: www.abcam.com/ab102524](http://www.abcam.com/ab102524)
(use www.abcam.cn/ab102524 for China, or www.abcam.co.jp/ab102524 for Japan)

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.

Table of Contents

1. Overview	1
2. Protocol Summary	2
3. Precautions	3
4. Storage and Stability	3
5. Limitations	4
6. Materials Supplied	4
7. Materials Required, Not Supplied	5
8. Technical Hints	6
9. Reagent Preparation	7
10. Standard Preparation	9
11. Sample Preparation	10
12. Assay Procedure	12
13. Calculations	14
14. Typical data	16
15. Quick Assay Procedure	17
16. Troubleshooting	18
17. Interferences	20
18. FAQs	20
19. Notes	21

1. Overview

Lipase Activity Assay Kit (Colorimetric) (ab102524) hydrolyzes a triglyceride substrate to form glycerol which is quantified enzymatically by via monitoring a linked change in the OxiRed probe absorbance (OD 570nm). This assay is rapid, simple, sensitive, and reliable, as well as, suitable for high throughput activity screening of lipase. This kit detects lipase activity as low as 0.02 mU per well.

Lipases perform essential roles in the digestion, transport and processing of dietary lipids (e.g. fats and oils) in living organisms. In humans, pancreatic lipase is the key enzyme responsible for breaking down fats in the digestive system by converting triglyceride to monoglyceride and free fatty acid. Pancreatic lipase monitoring is also used to help diagnose Crohn's disease, cystic fibrosis and celiac disease. Damage to the pancreas can exhibit a 5 – 10-fold increase of serum lipase levels within 24 to 48 hours.

2. Protocol Summary

Standard curve preparation



Sample preparation



Add reaction mix



Incubate for 60 – 90 minutes at 37°C and measure absorbance (OD570 nm) in kinetic mode

**For kinetic mode detection, incubation time given in this summary is for guidance only*

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

Δ Note: Reconstituted components are stable for 2 months.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition (Before prep)	Storage Condition (After prep)
Assay Buffer V/Assay Buffer	25 mL	-20°C	-20°C
Enzyme Mix VI/Enzyme Mix (lyophilized)	1 vial	-20°C	-20°C
Glycerol Standard/Glycerol Standard (100 mM)	200 µL	-20°C	-20°C
Lipase Positive Control/Lipase Positive Control (lyophilized)	1 vial	-20°C	-20°C
Lipase Substrate III/Lipase Substrate	400 µL	-20°C	-20°C
OxiRed Probe/OxiRed (in DMSO)	200 µL	-20°C	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 570 nm
- MilliQ water or other type of double distilled water (ddH₂O)
- PBS
- Calcium (1 – 5 mM)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom
- Dounce homogenizer (if using tissue)

8. Technical Hints

- This kit is sold based on number of tests. A “test” simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Assay Buffer V/Assay Buffer:

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 Enzyme Mix VI/Enzyme Mix (lyophilized):

Reconstitute the enzyme mix in 200 µL Assay Buffer V/Assay Buffer. Aliquot enzyme mix so that you have enough to perform the desired number of assays. Store at -20°C. Once reconstituted, use within 2 months.

9.3 Glycerol Standard/Glycerol Standard (100 mM):

Ready to use as supplied. Aliquot standard so that you have enough to perform the desired number of assays. Store at -20°C.

9.4 Lipase Positive Control/Lipase positive control (lyophilized):

Reconstitute the Lipase in 100 µL Assay Buffer V/Assay Buffer. Aliquot control so that you have enough to perform the desired number of assays. Store at -20°C. Once reconstituted, use within 2 months. Keep on ice while in use.

Immediately prior use, dilute 15 µL of positive control in 135 µL Assay Buffer V/Assay Buffer for use in the assay. Do not store diluted lipase.

9.5 Lipase Substrate III/Lipase Substrate:

Ready to use as supplied. Freezing may cause the substrate to separate from the aqueous phase. To re-dissolve the substrate, keep the cap tightly closed, then thaw in a hot water bath at 80 - 100°C for 1 minute until the substrate looks cloudy. Vortex for 30 seconds. The substrate should be clear. Repeat heat and vortex cycle one more time – the substrate is now completely in solution and ready to use.

Aliquot substrate so that you have enough volume to perform the desired number of assays.

Δ Note: heat/vortex cycle might need to be done every time to use a new aliquot of the Lipase substrate.

9.6 OxiRed Probe/OxiRed Probe (in DMSO):

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 min to thaw the DMSO solution before use.

Δ Note: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for a few minutes at 37°C.

Aliquot probe so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light. Once the probe is thawed, use within two months.

Δ Note: heating of the Probe might need to be done every time to use a new aliquot of the Probe.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

10.1 Prepare a 1 mM Glycerol standard by diluting 10 μL of the provided Glycerol Standard/100 mM Glycerol Standard (Step 9.3) with 990 μL of Assay Buffer V/Assay Buffer. Mix well by pipetting up and down.

10.2 Using the 1 mM standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Glycerol Standard (μL)	Assay Buffer V/Assay Buffer (μL)	Final volume standard in well (μL)	End amount glycerol in well (nmol/well)
1	0	150	50	0
2	6	144	50	2
3	12	138	50	4
4	18	132	50	6
5	24	126	50	8
6	30	120	50	10

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 μL).

11. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice and proceed with the Sample Preparation step. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples:

- 11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 2×10^6 cells).
- 11.1.2 Wash cells with cold PBS.
- 11.1.3 Resuspend cells in 100 μ L of Assay Buffer V/Assay Buffer.
- 11.1.4 Homogenize cells quickly by pipetting up and down a few times.
- 11.1.5 Centrifuge sample for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
- 11.1.6 Collect supernatant and transfer to a new tube.
- 11.1.7 Keep on ice.

11.2 Tissue samples:

- 11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 40 mg).
- 11.2.2 Wash tissue in cold PBS.
- 11.2.3 Resuspend tissue in 100 μ L of Assay Buffer V/Assay Buffer.
- 11.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.
- 11.2.5 Centrifuge samples for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
- 11.2.6 Collect supernatant and transfer to a new tube.
- 11.2.7 Keep on ice.

11.3 Plasma, Serum and Urine (and other biological fluids):

Lipid samples can be assayed directly or after dilution in the Assay Buffer V/Assay Buffer.

Δ Note: We suggest using different volumes of sample to ensure readings are within the standard curve range.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.
- Some lipases require calcium. If your lipase requires calcium, avoid EGTA in sample preparation and add calcium (1 – 5 mM) to Assay Buffer V/the lipase assay buffer before use.

Δ Note: Glycerol present in the sample can generate background in this assay. If you suspect your samples contain glycerol, set up Sample Background Controls.

12.1 Plate Loading:

- Standard wells = 50 μ L standard dilutions.
- Sample wells = 1 – 50 μ L samples (adjust volume to 50 μ L/well with Assay Buffer V/Assay Buffer).
- Sample Background Control wells = 1 – 50 μ L samples (adjust volume to 50 μ L/well with Assay Buffer V/Assay Buffer).
- Lipase positive control = 50 μ L diluted Lipase (Step 9.4)

12.2 Assay Reaction:

- 12.2.1 Prepare 100 μ L of Reaction Mix for each reaction. Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction mix to ensure consistency.

Component	Reaction Mix (μ L)	Background Reaction Mix (μ L)
Assay Buffer V/Assay Buffer	93	96
OxiRed Probe	2	2
Enzyme Mix VI/Enzyme Mix	2	2
Lipase Substrate III/Lipase Substrate	3	0

12.2.2 Add 100 µL of Reaction Mix into each standard, positive control and sample wells.

12.2.3 Add 100X µL of Background Reaction Mix into the background control sample wells.

12.2.4 Mix thoroughly.

12.3 Measurement:

12.3.1 Measure output immediately at OD 570 nm on a microplate reader in kinetic mode, every 2 – 3 minutes, for at least 60 - 90 minutes (if lipase activity is low) at 37°C protected from light.

Δ Note: Incubation time depends on the lipase activity in the samples. We recommend measuring OD in a kinetic mode, and choosing two time points (T1 and T2) to calculate the lipase activity of the samples. For standard curve, do not subtract A2 from A1. Standard curve can also be read in end point mode (i.e. at the end of incubation time).

13. Calculations

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.
- Use only the linear rate for calculation.

13.1 Standard curve calculation:

- 13.1.1 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
- 13.1.2 Average the duplicate reading for each standard.
- 13.1.3 Plot standard curve readings and draw the line of the best fit to construct the standard curve (most plate reader software or Excel can do this step). Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

13.2 Measurement of lipase in the sample:

- 13.2.1 For all reaction wells (including background control samples), choose two time points (T1 and T2) in the linear phase of the reaction progress curves and obtain the corresponding OD values at those points (OD1 and OD2)
- 13.2.2 Calculate ΔOD for sample as follows:

$$\Delta OD_{570nm} = OD2 - OD1$$

- 13.2.3 Determine the background corrected change in fluorescence intensity for each well of sample by subtracting the ΔOD value of the background control (BC).
- 13.3 Lipase activity (nmol/min/mL or mU/mL) in the test samples is calculated as:

$$\text{Lipase Activity} = \left(\frac{B \times D}{\Delta T \times V} \right)$$

Where:

B = amount of glycerol in sample well calculated from standard curve (nmol).

ΔT = linear phase reaction time T2 – T1 (minutes).

V = original sample volume added into the reaction well (mL).

D = sample dilution factor.

Unit definition:

1 Unit Lipase activity = amount of lipase that hydrolyzes triglyceride to generate 1.0 μmol of glycerol per minute at 37°C.

14. Typical data

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

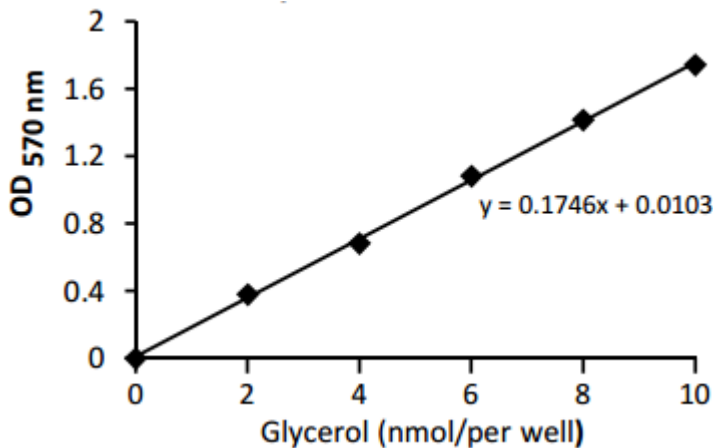


Figure 1. Typical glycerol standard calibration curve.

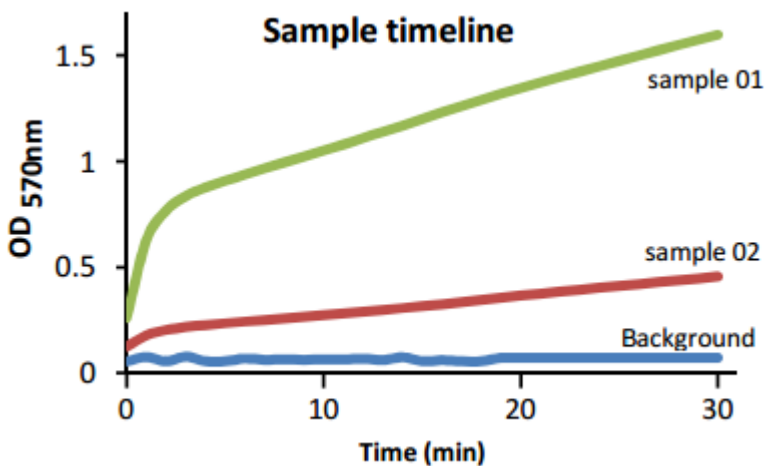


Figure 2. Time course curve of different samples.

15. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare glycerol standard, positive control, probe and prepare enzyme mix (aliquot if necessary); get equipment ready.
- Prepare glycerol standard dilution [2 – 10 nmol/well].
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (50 μ L), samples (50 μ L), positive control (50 μ L) and background sample control wells (50 μ L).
- Prepare a master mix for 50 μ L Reaction Mix and (if appropriate) a master mix for Background Reaction Mix:

Component	Reaction Mix (μ L)	Background Reaction Mix (μ L)
Assay Buffer V/Assay Buffer	93	96
OxiRed Probe	2	2
Enzyme Mix VI/Enzyme mix	2	2
Lipase Substrate III/Lipase Substrate	3	0

- Add 100 μ L Reaction to standard and sample wells.
- Add 100 μ L Background Reaction Mix to Sample Background control wells.
- Measure plate at OD 570 nm on a microplate reader in a kinetic mode at 37°C for 60 – 90 minutes protected from light.

16.Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Reason	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. Interferences

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:

- RIPA buffer: contains SDS which can destroy/decrease the activity of the enzyme.
- EGTA: if lipase in your sample requires calcium addition, do not add EGTA to sample.

18. FAQs

Q. What is the difference between this assay and Lipase Activity Assay Kit II (ab102525) and Lipase Activity Assay Kit III (ab118969)?

A. The lipase substrate used in each kit is different.

This assay (ab102524) uses a long-chain substrate; ab102525 uses a mid-length-chain substrate, whereas ab118969 uses a short-chain substrate.

19. Notes

Technical Support

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