ab102524 – Lipase Activity Assay Kit (Colorimetric)

For rapid, sensitive and accurate measurement of lipase activity levels in various samples.
For research use only - not intended for diagnostic use.

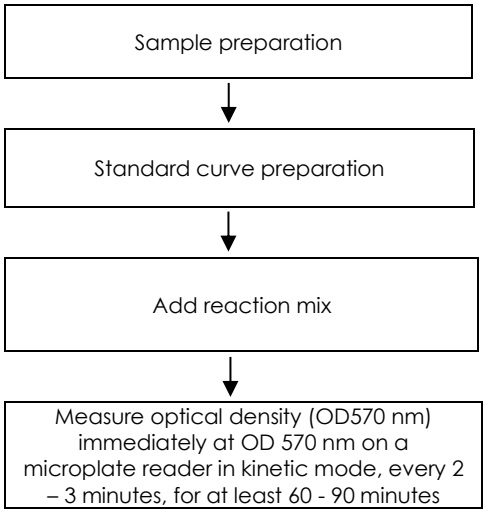
Background:

Abcam's Lipase Activity Assay Kit is a rapid, simple and sensitive assay for determining lipase activity in plasma, serum, cell culture media, cell culture supernatant, cell culture extracts, and tissue extracts.

In the assay, lipases hydrolyze a triglyceride substrate to form glycerol, which is quantified enzymatically by monitoring the formation of a stable chromophore, detectable by absorbance (OD 570 nm). This kit detects lipase activity as low as 0.02 mU per well.

Assay Summary:

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



QUICK ASSAY PROCEDURE

- Preheat plate reader to 37 °C
- Solubilize Enzyme Mix VI and Lipase Positive Control, thaw Assay Buffer 5, Glycerol Standard, Lipase Substrate III and OxiRed™ Probe
- Prepare samples in duplicate
- Prepare standard curve
- Set up plate for standard (50 µL) and samples (50 µL).
- Prepare and add 50 µL Reaction Mix to each well.
- Measure plate immediately at OD 570 nm on a microplate reader in kinetic mode, every 2 – 3 minutes, for 60 - 90 minutes

Precautions & Limitations:

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.

- Modifications to the kit components or procedures may result in loss of performance.
- 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.

Storage and Stability:

Store kit at -20°C in the dark immediately upon receipt. Reconstituted components are stable for 2 months. Do not use kit or components if they have exceeded the expiry date.

Materials Supplied:

Item	Quantity	Storage Temperature (on receipt)	Storage temperature (reconstituted)
Assay Buffer 5	25 mL	-20°C	-20°C
Enzyme Mix VI	1 vial	-20°C	-20°C
Glycerol Standard	200 µL	-20°C	-20°C
Lipase Positive Control	1 vial	-20°C	-20°C
Lipase Substrate III	400 µL	-20°C	-20°C
OxiRed™ Probe	0.2 mL	-20°C	-20°C

PLEASE NOTE: Assay Buffer 5 was previously labelled as Assay Buffer V and Assay Buffer, and Enzyme Mix VI as Enzyme Mix (lyophilized). Also, Lipase Substrate III was previously labelled as Lipase Substrate, and OxiRed™ Probe as Lipase OxiRed Probe and OxiRed (in DMSO). The compositions have not changed.

Materials Required, Not Supplied:

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

- Microplate reader capable of measuring absorbance (OD) at 570 nm (colorimetric)
- 96 well clear plate with clear flat bottom (colorimetric assay)
- Orbital shaker
- Microcentrifuge
- Dounce homogenizer (if using cells or tissue)
- MilliQ water or other type of double distilled/deionized water (ddH2O)
- 1 x PBS, pH 7.4 (ab285410 or similar)
- Calcium (1 – 5 mM)

Reagent Preparation:

- Briefly centrifuge small vials at low speed prior to opening.
- Equilibrate reagents to room temperature before use.
- Aliquot reagents so that you have enough volume to perform the desired number of assays.

Assay Buffer 5: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

Enzyme Mix VI: Reconstitute the enzyme mix in 220 µL Assay Buffer 5. 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.

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

Lipase Positive Control: Reconstitute the Lipase Positive Control in 100 µL Assay Buffer 5. 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.

Lipase Substrate III: 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.

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

OxiRed™ Probe: Ready to use as supplied. Warm the DMSO solution at 37 °C until completely thawed (~ 5 minutes), vortex briefly to create a homogenous solution, and spin down briefly to collect any contents trapped in the cap or seal. Keep at room temperature during use and reseal promptly to minimize moisture exposure.
For multiple use aliquot and store at −20 °C, protected from light and moisture. Stable for up to 2 months after thawing (≤3 freeze–thaw cycles).

Sample Preparation:

- We recommend performing several dilutions of your sample or testing multiple doses to ensure the readings are within the standard value range.

1. We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples at -80°C. Alternatively, 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. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.
2. Interferences:
   - RIPA buffer: contains SDS which can destroy/decrease the activity of the enzymes in the cascade.
   - EDTA/EGTA: if lipase in your sample requires calcium addition, do not add EDTA, EGTA (or other metal chelators) to sample. For example, use plasma treated with sodium/lithium heparin, rather than EDTA.

Cells (adherent or suspension) samples:
1. Harvest the number of cells necessary for each assay (initial recommendation = 2 x 10^6 cells).
2. Wash cells in cold PBS.
3. Resuspend cells in 100 µL of Assay Buffer 5.
4. Homogenize cells with a Dounce homogenizer sitting on ice.
5. Centrifuge sample for 2-5 minutes at 4° C at top speed using a cold microcentrifuge to remove any insoluble material.
6. Collect supernatant and transfer to a clean tube.
7. Keep on ice.

Tissue Samples:
1. Harvest the amount of tissue necessary for each assay (initial recommendation = 40 mg).
2. Wash tissue in cold PBS.
3. Resuspend tissue in 100 µL of Assay Buffer 5.
4. Homogenize tissue with a Dounce homogenizer sitting on ice with 10 - 15 passes.
5. Centrifuge samples for 2 - 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
6. Collect supernatant and transfer to a clean tube.
7. Keep on ice.

Liquid Samples (plasma, serum and other biological fluids): Samples can be used directly or diluted in Assay Buffer 5 for testing.

Standard Preparation:

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.
- Each dilution has enough standard to set up duplicate readings (2 x 50 µL).

1. Prepare a 1 mM dilution of Glycerol Standard as follows:1 mM dilution: Add 10 µL of the provided Glycerol Standard to 990 µL of Assay Buffer 5. Gently pipette up and down a few times to ensure all standard is removed from tip. Mix well by inversion
2. Add 0, 2, 4, 6, 8 and 10 µl of reconstituted 1mM Glycerol Standard in duplicate in 96 well plate and adjust volume to 50 µL with Assay Buffer 5 to generate 0, 2, 4, 6, 8, and 10 nmol/well of glycerol. or prepare standard curve dilution as described in the table below in a microplate or microcentrifuge tubes (sufficient for 2 replicate curves):

Standard #	Volume of 1 mM Glycerol Standard (µL)	Assay Buffer 5 (µL)	Final volume standard in well (µL)	End Glycerol Amount (nmol/well)
1	0	125	50	0
2	5	120	50	2
3	10	115	50	4
4	15	110	50	6
5	20	105	50	8
6	25	100	50	10

Assay Procedure:

- Keep enzymes and heat labile components and samples on ice during the assay.
- Equilibrate all other materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls, and samples in duplicate.
- Some lipases require calcium. If your lipase requires calcium, avoid EGTA in sample preparation and add calcium (1 – 5 mM) to Assay Buffer 5/the lipase assay buffer before use.

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

1. Set up Reaction wells:
   - Standard wells = 50 µL standard dilutions.
   - Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 5).
   - Sample Background wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 5).
   - Lipase Positive Control = 5 µL Lipase Positive Control (adjust volume to 50 µL/well with Assay Buffer 5).
2. Each well (standards, samples, and controls) requires 100 µL of Reaction Mix as shown in the table below. To ensure consistency, use the table below to prepare a Master Mix of the appropriate Reaction Mix for your assay using the following calculation:

   X µL component x (Number reactions +1).

Component	Colorimetric Assay Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer 5	93	96
OxiRed™ Probe	2	2
Enzyme Mix VI	2	2
Lipase Substrate III	3	0

3. Mix Master Reaction Mix by inversion. Add 100 µL of the Master Reaction Mix containing lipase substrate III to each standard, positive control and sample well. Use a clean tip for each well.
4. Add 100 µL of Background Reaction Mix into the background control sample wells. These wells should not contain Lipase Substrate III.
5. Measure output immediately at OD 570 nm on a microplate reader in kinetic mode, every 2 – 3 minutes, for at least 60 - 90 minutes (or longer if lipase activity is low) at 37°C protected from light.

   *Note: Incubation time depends on the lipase activity in the samples. We recommend measuring OD in a kinetic mode and choosing two time points (T_1 and T_2) to calculate the lipase activity of the samples. Standard curve can also be read in end point mode (i.e. at the end of incubation time).

Calculations:

- For samples producing signals greater than that of the highest standard: dilute further in appropriate buffer and reanalyze. Multiply the concentration found by the appropriate dilution factor.
- Use only the linear rate for calculation.

1. Average the duplicate reading for each standard and sample.
2. Subtract the mean absorbance value of the blank (Standard #1) from all standard. This is the corrected absorbance.
3. Plot the corrected absorbance values for each standard as a function of the amount of glycerol (in nmoles).
4. Find the equation of the corrected standard curve using a linear regression.
5. For all reaction wells (including background control samples), choose two time points (T_1 and T_2) in the linear phase of the reaction progress curves and obtain the corresponding OD values at those points (OD_1 and OD_2)
6. Calculate ΔOD for sample as follows:

   $$\Delta OD_{570nm} = OD_2 - OD_1$$

   Note: As assay is run at 37°C, it may take a few minutes for sample to reach the required temperature. Choose time points accordingly.

7. Determine the background corrected change in absorbance for each well of sample by subtracting the ΔOD value of the background control (BC) from the ΔOD value of the sample.

8. Use the $\Delta OD_{corrected}$ to obtain B nmol of glycerol generated by lipase during the reaction time from the standard curve.

   $$B = \left(\frac{\Delta OD_{corrected} - (y - intercept)}{Slope}\right)$$

9. Lipase activity (nmol/min/mL or mU/mL) in the test samples is calculated as:

   $$Lipase\ Activity = \left(\frac{B}{\Delta T * V}\right) * D = nmol/(min * mL) = mU/mL$$

   Where:

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

   ΔT = linear phase reaction time $T_2 - T_1$ (in minutes).

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

   D = sample dilution factor (before adding to plate).

   Unit definition:

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

Technical Support

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