ab185434

Lipolysis Assay Kit (Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Glycerol in cell cultures.

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.

Version 3a. Last Updated 24 August 2023

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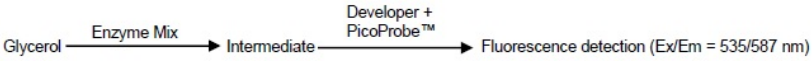
1. Overview

Lipolysis is the hydrolysis of triglycerides within the cell into glycerol and free fatty acids. The glycerol and free fatty acids are then released into the bloodstream or culture media. Lipolysis occurs in essentially all cells, but is most abundant in white and brown adipose tissue. Deficiencies in lipolysis lead to increased intracellular lipid accumulation, resulting in abnormal cellular physiology, hyperlipidemia, and insulin resistance. Lipolysis can be induced by catecholamine and certain hormones. The kit includes the synthetic catecholamine, Isoproterenol, which activates β-adrenergic receptors. This leads to activation of adenylate cyclase, which catalyzes the conversion of ATP to cAMP. cAMP then serves as a second messenger to activate hormone-sensitive lipase, which hydrolyzes the triglycerides. This pathway can be inhibited by insulin.

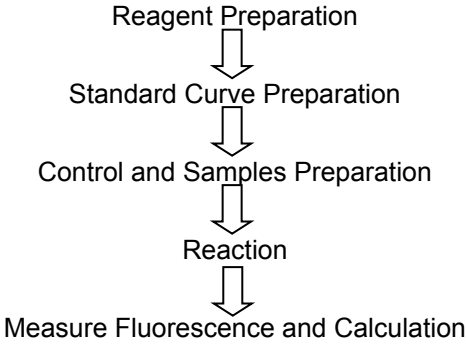
Abcam's Lipolysis Assay Kit (Fluorometric) (ab185434) is simple, easy-to-use and the most sensitive kit on the market. This assay measures glycerol released from 3T3-L1 cells as early as 1 hour after induction of lipolysis. It is suitable for measuring trace amounts of glycerol from samples. In this assay, glycerol reacts with Glycerol Enzyme Mix to form an intermediate that in turn reacts with Development Enzyme VI/Glycerol Developer and PicoProbe™ to generate the fluorescence product. The fluorescence intensity is directly proportional to the amount of glycerol. This assay kit can

detect less than 20 pmol of glycerol.

Figure 1: Assay Procedure



2. Protocol Summary



3. Kits Components

Item	Quantity
Glycerol Assay Buffer	25 mL
PicoProbe I/PicoProbe™	0.4 mL
Glycerol Enzyme Mix	1 vial
Isoproterenol/Isoproterenol (10 mM)	50 µL
Glycerol Standard/Glycerol Standard (100 mM)	0.2 mL
Development Enzyme Mix VI/Glycerol Developer	1 vial
Buffer IV/Lipolysis Assay Buffer	17 mL
Wash Buffer VIII/Lipolysis Wash Buffer	22 mL

4. Storage and Stability

Upon arrival, store the kit at-20°C and protect from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Briefly centrifuge all small vials prior to opening.

5. Materials Required, Not Supplied

- Distilled water (dH_2O)
- 96-well white or black plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

6. Reagents Preparation

1. **Glycerol Assay Buffer:**
   Warm Assay Buffer to room temperature before use. Store at -20°C.

2. **PicoProbe I/PicoProbe™:**
   PicoProbe I/PicoProbe™ is light sensitive and supplied ready to use. Warm to room temperature before use. Aliquot and store at -20°C. Stable for two months.

3. **Glycerol Enzyme Mix and Development Enzyme VI/Developer:**
   Reconstitute with 220 µL Glycerol Assay Buffer. Pipette gently to dissolve. Aliquot and store at -20°C. Keep on ice while in use. Stable for two months.

4. **Isoproterenol:**
   Warm to room temperature before use. Dilute the 10 mM stock solution 1/1000 in dH_2O to make a 10 µM working solution, as needed. Store at -20°C. Use within two months.

5. **Buffer IV/Lipolysis Assay Buffer and Wash Buffer:**
   Warm to 37°C before use. Store at 4°C or -20°C. Use within two months.

7. Assay Protocol

1. Sample Preparation

Grow and differentiate 3T3-L1 cells in a 96-well cell culture plate. After differentiation (lipid droplets should be visible by light microscopy), gently wash cells 2 times with 100 µL of Wash Buffer VIII/Lipolysis Wash Buffer. Remove wash buffer and replace with 150 µL Buffer IV/Lipolysis Assay Buffer, add 1.5 µL of 10 µM Isoproterenol (final concentration 100 nM) to wells to stimulate lipolysis. Stimulate lipolysis by incubation in 37°C tissue culture incubator for 1-3 hours. Collect media. Add 2-50 µL of media into 96-well plate and adjust the volume to 50 µL with Buffer IV/Lipolysis Assay Buffer.

Optional: Cells can be lysed and cell lysates can be used to normalize glycerol to cellular protein content using a BCA Protein Quantitation Kit (ab102536) or to a triglyceride level using a Triglyceride Quantification Kit (ab65336).

NOTE:

- *For unknown samples, we suggest performing pilot experiment and testing several doses of your samples to ensure the readings are within the Standard Curve range.*
- *Care should be taken while washing differentiated cells as differentiated cells are fragile and liable to detach with vigorous washing.*

- *As phenol red can affect the OD measurement, phenol red-free medium can be used, or the input volume of phenol red-containing medium can be limited to 20 µl per assay well.*
- *Higher concentrations of Isoproterenol interfere with the assay. If using a higher concentration or measuring larger sample volume, we recommend to spike the Standards with the same amount of Isoproterenol as used to stimulate the lipolysis and prepare Standard Curve.*

2. Standard Curve Preparation:

a) Dilute 100 mM Glycerol Standard (100 nmol/µL) to 1mM (1000 pmol/µL) by adding 10 µL of 100 mM Glycerol Standard to 990 µL Glycerol Assay Buffer and mix well. Further dilute the Glycerol Standard to 80 pmol/µL by adding 80 µL of 1mM Glycerol Standard to 920 µL Glycerol Assay Buffer and mix well.

b) Add 0, 2, 4, 6, 8 and 10 µL of 80 pmol/µL Glycerol Standard into series of wells in a 96-well plate to generate 0, 160, 320, 480, 640 and 800 pmol/well Glycerol Standards.

c) Adjust the volume to 50 µL/well with Glycerol Assay Buffer.

3. Reaction Mix:

Prepare enough Reaction Mix for the number of assays to be performed. For each well, prepare 50 µL Mix containing:

	Reaction Mix
Glycerol Assay Buffer	42 µL
Glycerol Enzyme Mix	2 µL
Development Enzyme Mix VI/Glycerol Developer	2 µL
PicoProbe I/PicoProbe™	4 µL

Add 50 µL of the reaction mix to each well containing the Standards, and test samples. Mix well.

4. Measurement

a) Incubate the plate at room temperature for 60 minutes protected from light.

b) Measure fluorescence (Ex/Em = 535/587 nm) in a microplate reader.

8. Data Analysis

Calculations:

a) Subtract 0 Standard reading from all readings. Plot the Standard Curve. Apply the corrected sample reading to the Standard Curve to get B pmol of Glycerol amount in the sample wells.

Sample Glycerol Concentration: C = B x T/S = pmol/well

Where:
B is the amount of Glycerol in the Standard Curve (pmol)
T is the total volume of the sample (µL).
S is the sample volume added into the reaction well (µL)

Glycerol molecular weight: 92.09 g/mol.

Alternatively, Glycerol concentration can also be expressed as pmol/µg protein or pmol/µg lipid.

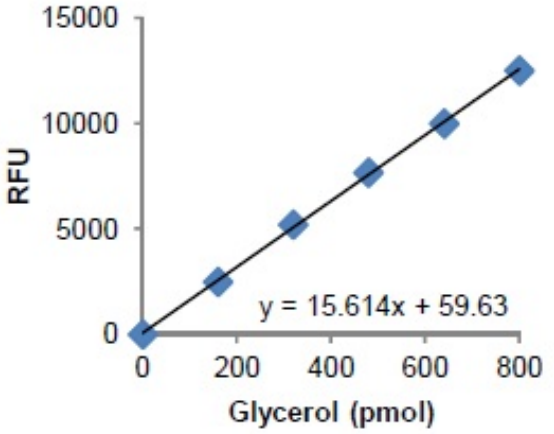


Figure 2. Glycerol Standard Curve.

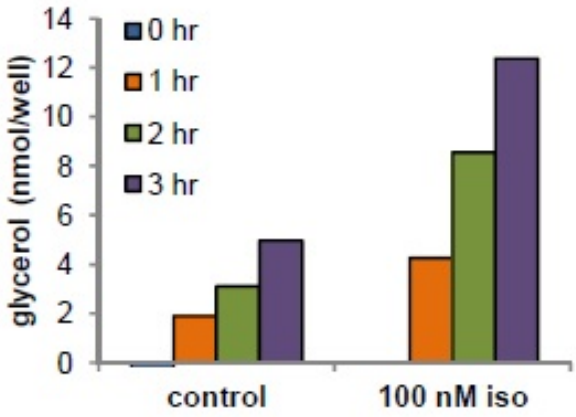


Figure 3. Measurement of Glycerol Level in media (5 µl) of 3T3-L1 cells treated with vehicle control (H_2O) or 100 nM Isoproterenol for 0-3 hours. Measurements for 0 hr. were undetectable.

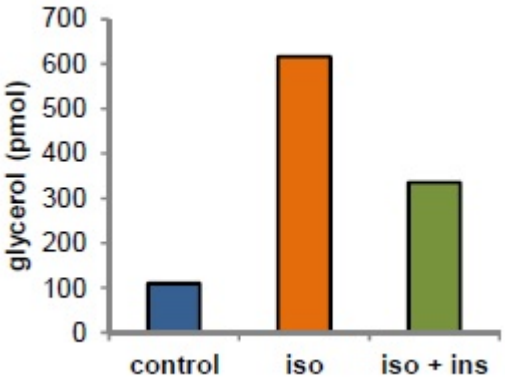


Figure 4. Inhibition of Isoproterenol (100 nM) stimulated lipolysis by treatment with 100 nM insulin (measured using 5 µl of media). Control was vehicle (H_2O) treated cells.

9. 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

Problem	Reason	Solution
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)