

ab185433

Lipolysis Assay Kit (Colorimetric)

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.

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1. Overview

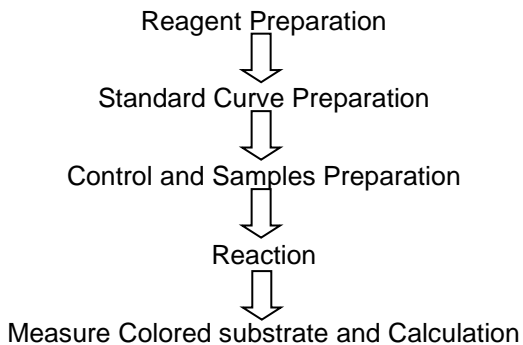
Lipolysis is the hydrolysis of triglycerides within the cell into glycerol and free fatty acids. The glycerol and free fatty acids are then released into the bloodstream or culture media. Lipolysis occurs in essentially all cells, but is most abundant in white and brown adipose tissue. Deficiencies in 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 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 (Colorimetric) (ab185433) is simple and easy-to-use. The assay measures glycerol released from 3T3-L1 cells after induction of lipolysis using colorimetric method. The color intensity is directly proportional to the amount of glycerol. This assay kit can detect less than 200 pmol of glycerol.

Figure 1: Assay Procedure



2. Protocol Summary



3. Kits Components

Item	Quantity
Assay Buffer 5	25 mL
OxiRed™ Probe	0.2 mL
Enzyme Mix VI	1 vial
Isoproterenol	50 µL
Glycerol Standard	200 µL
Buffer IV	17 mL
Wash Buffer VIII	22 mL

PLEASE NOTE: Assay Buffer 5 was previously labelled as Assay Buffer V and Glycerol Assay Buffer, and Enzyme Mix VI as Glycerol Enzyme Mix (Lyophilized), and OxiRed™ Probe as OxiRed Probe and Glycerol Probe. The composition has not changed.

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.

4. Materials Required, Not Supplied

- Distilled water (dH₂O)
- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- Centrifuge with cooling option

5. Reagents Preparation

1. Assay Buffer 5:

Aliquot and store at -20°C. Warm to room temperature before use.

2. OxiRed™ Probe:

Briefly warm at 37°C for 1-2 minutes to dissolve. Mix well. Store at -20°C. Use within 2 months.

3. Enzyme Mix VI:

Reconstitute with 220 µL Assay Buffer 5. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Use within two months.

4. Isoproterenol:

Warm to room temperature before use. Dilute the 10 mM stock solution 1/1,000 in dH₂O to make a 10 µM working solution as needed. Store at -20°C. Use within two months.

5. Buffer IV and Wash Buffer VIII:

Warm to 37°C before use. Store at 4°C or -20°C.

6. 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 using microscopy), gently wash cells two times with 100 μ L of Wash Buffer VIII. Remove Wash Buffer VIII and replace with 150 μ L Buffer IV. Add 1.5 μ L of 10 μ M Isoproterenol (final concentration 100 nM) to wells to stimulate lipolysis. Stimulate lipolysis for 1-3 hours or longer if desired. Collect media. Add 20-50 μ L of media into 96-well plate and adjust the volume to 50 μ L with Buffer IV. Cells can be lysed and 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 (ab89001).

NOTE:

- *Care should be taken while washing differentiated cells as differentiated cells are fragile and liable to detach with vigorous washing*
- *For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.*
- *Higher concentrations of Isoproterenol interfere with the assay. If using a higher concentration or measuring larger*

sample volume we recommend spiking the Standards with the same amount of Isoproterenol as used to stimulate the lipolysis and prepare Standard Curve.

2. Standard Curve Preparation:

- a) Add 10 μL of 100 mM Glycerol Standard to 990 μL of Assay Buffer 5 to generate 1 mM Glycerol Standard and mix well.
- b) Add 0, 2, 4, 6, 8, and 10 μL of 1 mM Glycerol Standard into series of wells in 96-well plate to generate 0, 2, 4, 6, 8, and 10 nmol/well of Glycerol Standards.
- c) Adjust the volume to 50 μL per well with Assay Buffer 5.

3. Reaction Mix:

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

Reaction Mix	
Assay Buffer 5	46 μ L
Enzyme Mix VI	2 μ L
OxiRed™ Probe	2 μ L

Mix the Reaction Mix well and then add 50 μ L of the Reaction Mix to each well containing the Standards and test samples.

4. Measurement

- Incubate the plate at room temperature for 30 minutes protected from light.
- Measure absorbance @ OD 570 nm in a microtiter plate reader. The reaction is stable for at least 2 hours.

7. 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 nmol of Glycerol amount in the sample wells.

$$\text{Sample Glycerol Concentration: } C = B \times T/S = \text{nmol/well}$$

Where:

B = amount of glycerol from Standard Curve (nmol).

T = total volume of the sample (μL).

S = sample volume added into the reaction well (μL).

Glycerol molecular weight: 92.09 g/mol.

Glycerol can be expressed in nmol or nmol/well; alternatively as nmol/ μg protein or nmol/ μg lipid.

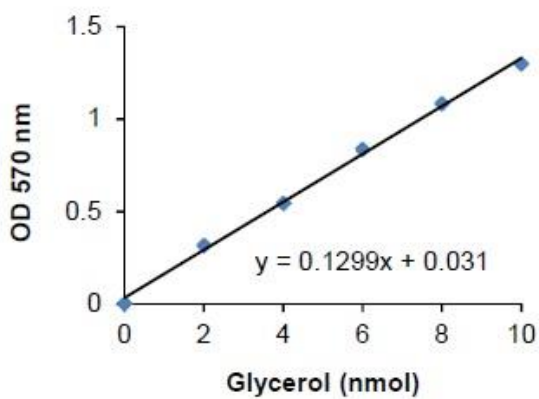


Figure 2. Glycerol Standard Curve.

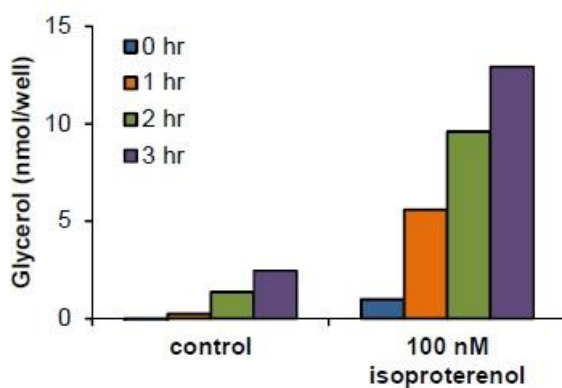


Figure 3. Measurement of glycerol level in media (50 μ L) of 3T3-L1 cells treated with vehicle control (H_2O) or 100 nM Isoproterenol for 0-3 hours.

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

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

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