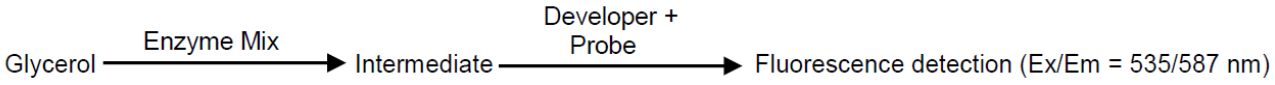
Lipolysis (3T3-L1) Assay Kit (Fluorometric)

LS-K877-100 (100 Tests) • **See Storage Conditions Below**

LSBio
LifeSpan BioSciences, Inc.

Introduction

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. LSBio's Lipolysis (3T3-L1) Fluorometric Assay Kit is simple and easy-to-use. 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 Enzyme Mix to form an intermediate that in turn reacts with Glycerol Developer & Probe 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.



Applications

- Measurement of lipolysis by 3T3-L1 cells or adipocytes.
- Screening compounds that influence lipolysis, mechanistic studies and studying metabolic dysfunctions.

Sample Types

- Primary adipocytes
- Cell culture: 3T3-L1 cells

Components

Component	K877-100 100 Tests	Cap Code
Lipolysis Assay Buffer	17 ml	NM
Lipolysis Wash Buffer	22 ml	NM/Brown
Glycerol Assay Buffer	25 ml	WM
Probe (in DMSO, anhydrous)	0.4 ml	Blue
Glycerol Enzyme Mix (Lyophilized)	1 vial	Green
Glycerol Developer (Lyophilized)	1 vial	Red
Glycerol Standard (100 mM)	0.2 ml	Yellow
Isoproterenol (10 mM)	50 µl	Violet

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Materials Not Supplied

- 96-well plate with flat bottom, preferably white or black plates.
- BCA Protein Quantitation Kit (LSBio, Cat. # LS-K706)
- Triglyceride Quantification Kit (LSBio, Cat. # LS-K126)

Storage Conditions and Reagents Preparation

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- Lipolysis Assay Buffer: Warm to 37°C before use. Store at 4°C or -20°C. Use within two months.
- Lipolysis Wash Buffer: Warm to 37°C before use. Store at 4°C or -20°C. Use within two months.
- Glycerol Assay Buffer: Warm to room temperature before use. Store at -20°C.
- Probe (in DMSO): Ready to use as supplied. Warm to room temperature before use. Store at -20°C. Use within 2 months.
- Glycerol Enzyme Mix and Developer: Reconstitute with 220 µl Glycerol Assay Buffer by gently pipetting up & down, making sure the material is completely dissolved. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Keep on ice while in use. Use within 2 months.
- Isoproterenol: Warm to room temperature before use. Dilute the 10 mM stock solution 1:1,000 in dH_2O to make a 10 µM working solution, as needed. Store at -20°C. Use within two months.

Assay Procedure

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 Lipolysis Wash Buffer. Remove wash buffer and replace with 150 µl Lipolysis Assay Buffer, add 1.5 µl of 10 µM Isoproterenol (final concentration 100 nM) to wells to stimulate lipolysis. Stimulate lipolysis for 1-3 hours. Collect media. Add 2-50 µl of media into 96-well plate & adjust the volume to 50 µl with Lipolysis Assay Buffer. Cells can be lysed and cell lysates can be used to normalize glycerol to cellular protein content using BCA Protein Quantitation Kit (LSBio, Cat. # LS-K706) or triglyceride level using Triglyceride Quantification Colorimetric/Fluorometric Assay Kit (LSBio, Cat. # LS-K126).

   Notes:

   a) For unknown samples, we suggest performing pilot experiment & testing several doses of your samples to ensure the readings are within the Standard Curve range.

   b) Care should be taken while washing differentiated cells as differentiated cells are fragile and liable to detach with vigorous washing.

   c) 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: Dilute 100 mM Glycerol Standard (100 nmol/µl) to 1 mM (1,000 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 1 mM Glycerol Standard to 920 µl Glycerol Assay Buffer and mix well. Add 0, 2, 4, 6, 8 & 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. Adjust the volume to 50 µl per well with Glycerol Assay Buffer.

3. Reaction Mix: Mix enough reagents for the number of assays (samples and standards) to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix
Glycerol Assay Buffer	42 µl
Probe	4 µl
Glycerol Enzyme Mix	2 µl
Glycerol Developer	2 µl

Mix well. Add 50 µl of Reaction Mix to each well containing the Standard and test samples. Mix well.

4. Measurement: Incubate the reaction for 60 min. at room temperature, protected from light. Measure fluorescence (Ex/Em = 535/587 nm) in a microplate reader.

5. Calculation: 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 = the amount of glycerol from the Standard Curve (pmol), T = the total volume of the sample (µl), S = 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.

Sample Data

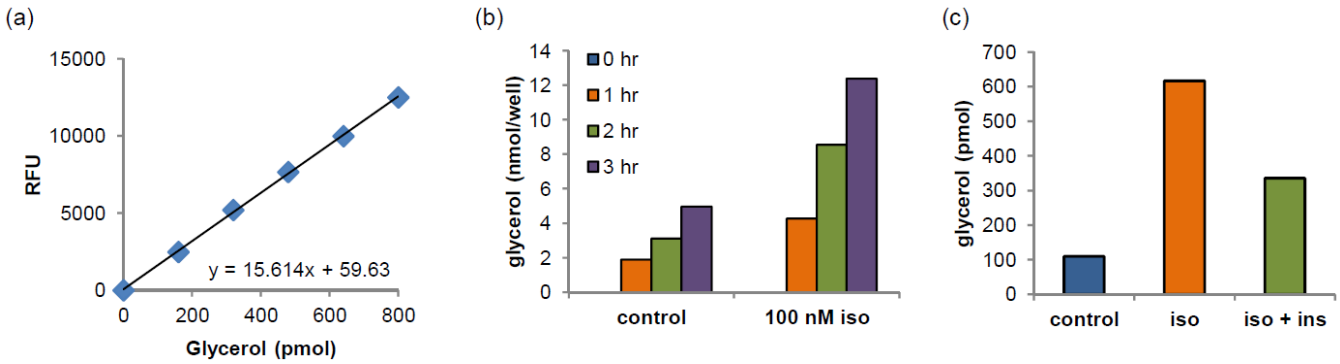

Figure: (a) Glycerol Standard Curve. (b) Measurement of Glycerol Level in media (5 µl) of 3T3-L1 cells treated with vehicle control (H_2O) or 100 nM Isoproterenol for 0-3 hours. Measurements for 0 hour were undetectable. (c) Inhibition of Isoproterenol (100 nM) stimulated lipolysis by treatment with 100 nM insulin (measured using 5 µl of media). Control was vehicle (H_2O) treated cells. Assays were performed following the kit protocol.

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