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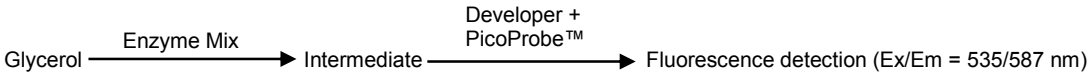
PicoProbe™ Lipolysis (3T3-L1) Fluorometric Assay Kit

rev. 11/13

(Catalog # K578-100; 100 assays; Store at -20°C)

I. 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. BioVision's Lipolysis (3T3-L1) Fluorometric Assay kit is simple, easy-to-use & the most sensitive kit on the market. This assay measures glycerol released from 3T3-L1 cells as early as 1 hr. 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 & 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.



II. Application:

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

III. Sample Type:

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

IV. Kit Contents:

Components	K578-100	Cap Code	Part Number
Lipolysis Assay Buffer	17 ml	NM	K578-100-1
Lipolysis Wash Buffer	22 ml	NM, brown	K578-100-2
Glycerol Assay Buffer	25 ml	WM	K578-100-3
PicoProbe™ (in DMSO, anhydrous)	0.4 ml	Blue	K578-100-4
Glycerol Enzyme Mix (Lyophilized)	1 vial	Green	K578-100-5
Glycerol Developer (Lyophilized)	1 vial	Red	K578-100-6
Glycerol Standard (100 mM)	0.2 ml	Yellow	K578-100-7
Isoproterenol (10 mM)	50 µl	Violet	K578-100-8

V. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom, preferably white or black plates.
- Multi-well spectrophotometer (ELISA reader)

VI. Storage and Handling:

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

VII. Reagent Preparation and Storage Conditions:

- **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.
- **PicoProbe™** (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 1:1000 in dH_2O to make a 10 µM working solution, as needed. Store at -20°C. Use within two months.

VIII. Glycerol 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 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 hr. 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 (Cat. # K812) or triglyceride level using Triglyceride Quantification Colorimetric/Fluorometric Assay Kit (Cat. # K622).
   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 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. 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
PicoProbe™	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.

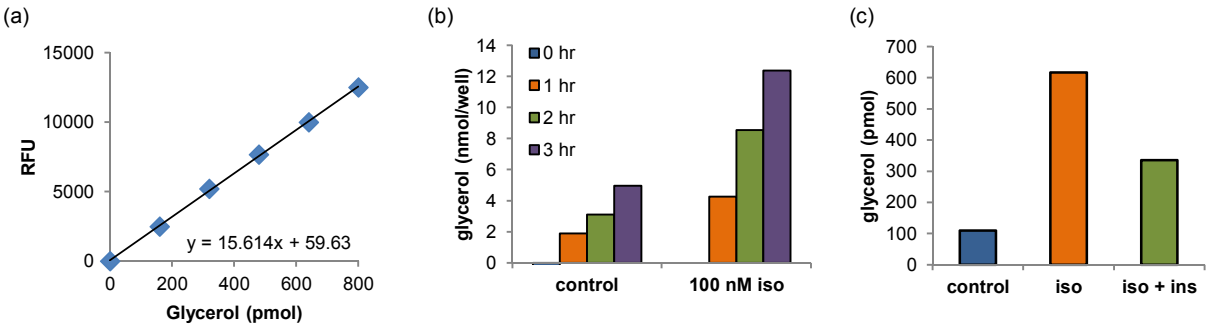


Figure: (a) Glycerol Standard Curve. (b) Measurement of Glycerol Level in media (5 µl) of 3T3-L1 cells treated with vehicle control (H_2O) or 100 nM Isoproterenol for 0-3 hr., Measurements for 0 hr. were undetectable. (c) Inhibition of Isoproterenol (100 nM) stimulated lipolysis by treatment with 100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

IX. RELATED PRODUCTS:

Lipolysis (3T3-L1) Colorimetric Assay Kit (K577)
3T3-L1 Differentiation Kit (K579)
Adipogenesis Colorimetric/Fluorometric Kit (K610)
Free Glycerol Colorimetric/Fluorometric Assay Kit (K630)
Free Glycerol Colorimetric Assay Kit II (K634)
PicoProbe™ Free Glycerol Fluorometric Assay Kit (K643)
Glucose Uptake Colorimetric Assay Kit (K676)
Glucose Uptake Fluorometric Assay Kit (K666)
Cell Lysis Buffer (1067)
Glucose Colorimetric/Fluorometric Assay Kit (K616)
Insulin (human) ELISA Kit (K4742)
Adiponectin (human) Elisa Assay Kit (K4901)
Adiponectin (mouse) Elisa Assay Kit (K4902)
Adiponectin (rat) Elisa Assay Kit (K4903)
Resistin (human/mouse/rat) EIA Kit (K4767)
Leptin (human) ELISA Kit (K4777)
Triglyceride Quantification Colorimetric/Fluorometric Assay Kit (K622)
BCA Protein Quantitation Kit (K812)

FOR RESEARCH USE ONLY! Not to be used on humans.