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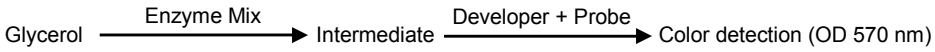
Lipolysis (3T3-L1) Colorimetric Assay Kit

rev. 1/14

(Catalog # K577-100; 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 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 3T3-L1 Lipolysis Assay kit 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.



II. Application:

- Measurement of lipolysis in 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	K577-100	Cap Code	Part Number
Lipolysis Assay Buffer	17 ml	NM	K577-100-1
Lipolysis Wash Buffer	22 ml	NM, brown	K577-100-2
Glycerol Assay Buffer	25 ml	WM	K577-100-3
Glycerol Probe (in DMSO, Anhydrous)	0.2 ml	Red	K577-100-4A
Glycerol Enzyme Mix (Lyophilized)	1 vial	Green	K577-100-5
Glycerol Standard (100 mM)	0.2 ml	Yellow	K577-100-6
Isoproterenol (10 mM)	50 µl	Violet	K577-100-7

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer

VI. Storage and Handling:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read 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.
- **Lipolysis Wash Buffer:** Warm to 37°C before use. Store at 4°C or -20°C.
- **Glycerol Assay Buffer:** Warm to room temperature before use. Store at -20°C.
- **Glycerol Probe:** Briefly warm at 37°C for 1-2 min. to dissolve. Mix well. Store at -20°C. Use within 2 months.
- **Glycerol Enzyme Mix**: 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. Keep on ice while in use. Use within two 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. Lipolysis 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 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. or longer if desired. Collect media. Add 20-50 µl of media into 96-well plate & adjust the volume to 50 µl with Lipolysis Assay Buffer. Cells can be lysed and 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 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 spiking the Standards with the same amount of Isoproterenol as used to stimulate the lipolysis and prepare Standard Curve.

2. **Standard Curve Preparation:** Add 10 µl of 100 mM Glycerol Standard to 990 µl of Glycerol Assay Buffer to generate 1 mM Glycerol Standard and mix well. Add 0, 2, 4, 6, 8, & 10 µl of 1 mM Glycerol Standard into series of wells in 96-well plate to generate 0, 2, 4, 6, 8, & 10 nmol/well of 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	46 µl
Glycerol Probe	2 µl
Glycerol Enzyme Mix	2 µl

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

4. **Measurement:** Incubate the plate at room temperature for 30 min. protected from light. Measure absorbance (OD 570 nm) in a microtiter plate reader. The reaction is stable for at least 2 hr.
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 nmol of Glycerol amount in the sample wells.

Sample Glycerol Concentration: C = B x T/S = 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.

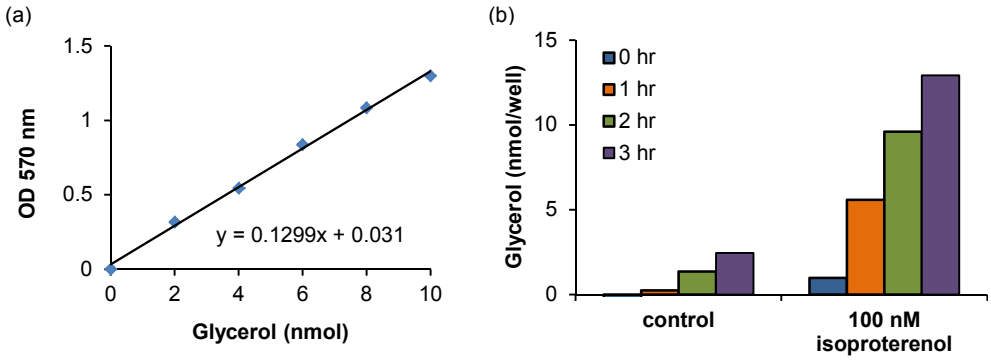


Figure: (a) Glycerol Standard Curve, (b) Measurement of glycerol level in media (50 µl) of 3T3-L1 cells treated with vehicle control (H_2O) or 100 nM Isoproterenol for 0-3 hr.

IX. RELATED PRODUCTS:

3T3-L1 Lipolysis Fluorometric Assay Kit (K578)
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)

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