

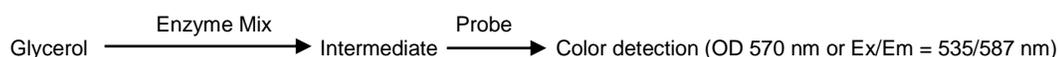
Lipolysis (Adipocyte) Colorimetric/Fluorometric Assay Kit

04/15

(Catalog # K581-5; for 5 g tissue; Store at -20°C)

I. Introduction:

Lipolysis is the intracellular hydrolysis of triglycerides into glycerol and free fatty acids, which 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 hormones or catecholamine, which binds to β -adrenergic receptors leading to activation of adenylyl cyclase, forming cAMP from ATP. cAMP serves as a second messenger to activate hormone-sensitive lipase, which hydrolyzes the triglycerides. This pathway is inhibited by insulin. BioVision's Adipocyte Lipolysis Colorimetric/Fluorometric Assay kit is a simple, facile way to monitor lipolysis. The kit provides all reagents necessary to isolate adipocytes from up to 5 g of mouse or rat adipose tissue and measure glycerol release from primary adipocytes after induction of lipolysis. It also includes the synthetic catecholamine, Isoproterenol, to stimulate the cAMP-mediated pathway. The signal intensity is directly proportional to the amount of glycerol present. This assay kit can detect less than 200 pmol glycerol in the colorimetric assay and less than 20 pmol in the fluorometric assay.



II. Application:

- Isolation of adipocytes from rat/mouse tissue
- Measurement of lipolysis by adipocytes
- Screening compounds that influence lipolysis, mechanistic studies and studies on metabolic dysfunction

III. Sample Type:

Animal tissues: adipocytes

IV. Kit Contents:

Components	K581-5	Cap Code	Part Number
Collagenase (0.2%)	10 ml	NM, blue	K581-5-1
Collagenase Stop Buffer	90 ml	NM	K581-5-2
Adipocyte Wash Buffer	22 ml	NM, brown	K581-5-3
Adipocyte Lipolysis Buffer	17 ml	NM	K581-5-4
Glycerol Assay Buffer	25 ml	WM	K581-5-5
Glycerol Probe (in DMSO, anhydrous)	0.2 ml	Red	K581-5-6
Glycerol Enzyme Mix (Lyophilized)	1 vial	Green	K581-5-7
Glycerol Standard (100 mM)	0.2 ml	Yellow	K581-5-8
Isoproterenol (10 mM)	50 μ l	Violet	K581-5-9
Cell strainer (100 μ m)	10	-	K581-5-10

V. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom (**Note:** Clear plates are preferred for colorimetric assay and black or white plates are preferred for fluorometric assay), 50 ml conical tubes, orbital shaker, dissecting scissors, 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 entire protocol before performing the assay.

VII. Reagent Preparation and Storage Conditions:

- **Collagenase (0.2%):** Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Use within two months.
- **Collagenase Stop Buffer:** Warm to 37°C before use. Store at 4°C or -20°C.
- **Adipocyte Wash and Adipocyte Lipolysis 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 μ l 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 dH₂O to make a 10 μ M working solution, as needed. Store at -20°C. Use within two months.

VIII. Adipocyte Lipolysis Protocol:

1. Sample Preparation:

- Isolation of Adipocytes:** Use freshly isolated adipose tissue from mice or rats. Mince thoroughly with dissecting scissors for 5 min. Place minced tissue in a 50 ml conical tube with cap loosely on and add 2 ml Collagenase (0.2%) per 1g tissue. Incubate in a heated orbital shaker at 37°C for 30 min. at 160 rpm. Add 9 ml of Collagenase Stop Buffer per 1 ml of Collagenase (0.2%) and mix by inverting. Filter through 100 μ m Cell Strainer and centrifuge filtrate at 500 x g for 10 min. Gently transfer the top layer (adipocytes) to a new conical tube.
- Stimulation of Lipolysis:** Wash adipocytes 2X with 2 ml Adipocyte Wash Buffer per 1g tissue. Centrifuge at 500 x g for 10 min. Remove Wash Buffer and add 1.5 ml Adipocyte Lipolysis Buffer per 1g tissue or as needed. Aliquot 150 μ l of adipocytes into separate tubes for each reaction & add 1.5 μ l of 10 μ M Isoproterenol (for final concentration of 100 nM) to samples to be stimulated for lipolysis (higher concentration not recommended due to isoproterenol interference with detection). Stimulate lipolysis for 1-3 hrs or

longer if desired by incubating at 37°C. Collect medium and add 20-50 µl for the colorimetric assay or dilute 1/10 and add 2-20 µl for the fluorometric assay into 96-well plate. Adjust the volume to 50 µl per well with Adipocyte Lipolysis 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:

- Important: Thorough mincing of tissue by scissors is crucial for proper tissue digestion.
- Tissue may require shorter or longer digestion time with Collagenase. If tissue is not completely digested, increase digestion time. In most instances, 20-45 min. digestion will be sufficient.
- For unknown samples, we suggest doing a pilot experiment & testing several doses of your samples to ensure readings are within the standard curve range.
- Isoproterenol $\geq 5 \mu\text{M}$ (0.5 nmol/well) interferes with the assay. If using a higher concentration or measuring a larger sample volume, we recommend to spike the Standard Curve with the same amount of Isoproterenol as used to stimulate lipolysis.

2. Standard Curve Preparation:

For colorimetric assay, add 10 µl of the Glycerol Standard to 990 µl of Adipocyte Lipolysis Buffer to generate 1 mM Glycerol Standard, mix well. Add 0, 2, 4, 6, 8 & 10 µl of the 1 mM Glycerol Standard into a series of wells in 96-well plate to generate 0, 2, 4, 6, 8 & 10 nmol/well of Standards. Adjust the volume to 50 µl per well with Adipocyte Lipolysis Buffer.

For fluorometric assay, add 20 µl of 1 mM Glycerol Standard to 980 µl of Adipocyte Lipolysis Buffer to generate 20 µM Glycerol Standard, mix well. Add 0, 2, 4, 6, 8 & 10 µl of the 20 µM Glycerol Standard into a series of wells in 96-well plate to generate 0, 40, 80, 120, 160 & 200 pmol/well of Standards. Adjust the volume to 50 µl per well with Adipocyte Lipolysis Buffer.

- 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 the Reaction Mix to each well containing the Standard and test samples and mix.

* For the fluorometric assay, use 0.4 µl/well of the Glycerol Probe to decrease the background readings.

- Measurement:** Incubate at room temperature for 30 min. protected from light. Measure absorbance (OD 570 nm) or fluorescence (Ex/Em = 535/587 nm). The reaction is stable for at least 2 hr.
- 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: $C = B \times T/S \times D = \text{nmol}/100 \text{ mg tissue}$

Where: **B** = amount of glycerol from Standard Curve (nmol)
T = total volume of the sample (150 µl)
S = sample volume added into reaction well (µl)
D = dilution factor

Glycerol molecular weight: 92.09 g/mole

Glycerol can be expressed in nmol or nmol/100 mg tissue; alternatively as nmol/µg protein or nmol/µg lipid.

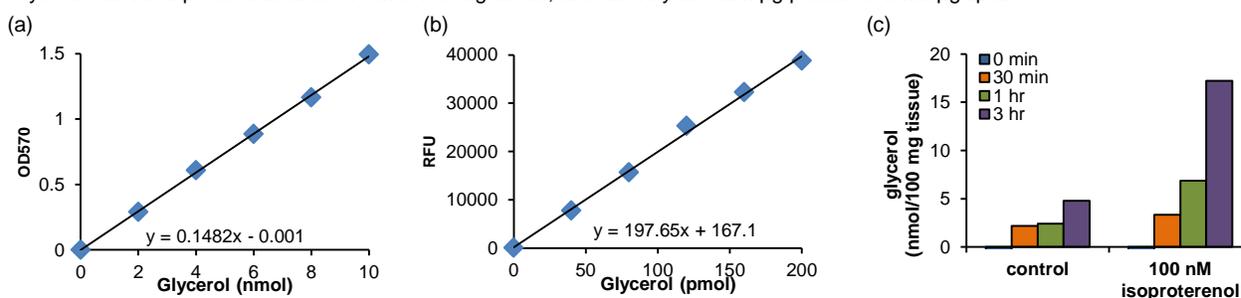


Figure: Glycerol Standard Curve: (a) colorimetric assay, (b) fluorometric assay & (c) Measurement of glycerol level from adipocyte samples (20 µl of 1/10 diluted sample) using fluorometric assay. Adipocytes were either treated with vehicle control (H₂O) or 100 nM isoproterenol for 0-3 hr.

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)
 Leptin (human) ELISA Kit (K4777)
 Cell Lysis Buffer (1067)

PicoProbe™ Lipolysis (3T3-L1) Fluorometric Assay Kit (K578)
 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)
 Triglyceride Quantification Colorimetric/Fluorometric Assay Kit (K622)
 BCA Protein Quantitation Kit (K812)

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