Adipolysis (Lipolysis) Assay Kit

Adipolysis (Lipolysis) Assay Kit is a detection kit for direct quantification of glycerol released during adipolysis and lipolysis in cell culture supernatants. This kit detects glycerol with single working reagent and incubation for 20 min at room temperature only.

Catalog number: ARG82130

Package: 200 tests

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Lipolysis is the metabolic pathway through which lipid triglycerides are hydrolyzed into a glycerol and three fatty acids. It is used to mobilize stored energy during fasting or exercise, and usually occurs in fat adipocytes. The most important regulatory hormone in lipolysis is insulin; lipolysis can only occur when insulin action falls to low levels, as occurs during fasting. Other hormones that affect lipolysis include glucagon, epinephrine, norepinephrine, growth hormone, atrial natriuretic peptide, brain natriuretic peptide, and cortisol. [Provide by Wikipedia: Lipolysis]

PRINCIPLE OF THE ASSAY

This Adipolysis (Lipolysis) Assay Kit is a simple colorimetric assay that measures the amount of glycerol release during lipolysis present in cell culture supernatant. This homogeneous assay uses a single Working Reagent that combine glycerol kinase, glycerol phosphate oxidase and color reactions in one step. The color intensity of the reaction product at O.D. 570 nm is directly proportional to glycerol concentration in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on ice. Store all components at -20°C. Shelf life of 12 months after receipt.

Component	Quantity	Storage information
Assay Buffer	24 mL	-20°C
Dye Reagent	220 µL	-20°C
Enzyme Mix	500 µL	-20°C
ATP	250 µL	-20°C
Standard (100 mM Glycerol)	100 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 570 nm
- Fluorescence microplate reader capable of reading excitation at 530 nm and emission at 585 nm.
- Centrifuge
- Clear or black flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- SH-group containing reagents (E.g., mercaptoethanol, DTT) may interfere with this assay and should be avoided in sample preparation.
- Cells and testing drugs are to be provided by the customer and are not included in this reagent kit.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell culture: Grow cells (E.g., preadipocytes, adipocytes) in culture plate (24-well, 96-well or 384-well). If desired, treat cells with testing drugs such as insulin, isoproterenol, and incubate for the desired time period.

Cell Culture Supernatants: Centrifuge cell culture media at 1,500 x g for 10 minutes at 4°C to remove particulates. Samples should be assayed immediately or stored at-20°C.

Note:

1. SH-group containing reagents (E.g., mercaptoethanol, DTT) may interfere with this assay and should be avoided in sample preparation.
2. Cells and testing drugs are to be provided by the customer and are not included in this reagent kit.

REAGENT PREPARATION

- **Working Reagent:** For each assay well, mix 100 µL of Assay Buffer, 2µL of Enzyme Mix, 1 µL of ATP and 1 µL of Dye Reagent in a clean tube. Mix well.
- **Standard:** Prepare a 100 µg/mL standard by mixing 10 µL of 100 mM glycerol standard with 910 µL of the same medium used for cell culture. Dilute standard in the medium as follows.

Standard tube	Final Glycerol conc. (µg/mL)	Medium	100 µg/mL standard
S1	100	0	400
S2	60	200	300
S3	30	350	150
S4	0	500	0

ASSAY PROCEDURE

Prior to the assay, equilibrate all components to room temperature. Keep thawed Enzyme Mix in a refrigerator or on ice during assays.

Colorimetric procedure:

1. Add **10 µL** of **Standards** or **sample** into wells of a clear 96-well microplate.
2. Add **100 µL** of **Working Reagent** into each well. Tap plate to mix.
3. Incubate for **20 minutes** at **room temperature**.
4. Read the absorbance at **O.D. 570 nm.**

Note: if the Sample OD is higher than the Standard OD at 100 µg/mL, dilute sample in water and repeat the assay. Multiply result by the dilution factor.

Fluorimetric procedure:

For fluorimetric assays, the linear detection range is 0.2 to 5 µg/mL of glycerol. Dilute Standards (S1 to S4, see REAGENT PREPARATION): mix 10 µL of standard with 190 µL of distilled water. The glycerol concentrations are now 5.0, 3.0, 1.5 and 0 µg/mL, respectively.

1. Transfer **5 µL** of diluted **Standard** and **samples** into wells of a black 96-well microplate.
2. Add **50 µL** of **Working Reagent** into each well. Tap plate to mix.
3. Incubate for **20 minutes** at **room temperature**.
4. Read the plate with a fluorescence microplate reader using 530 nm excitation filter and nm emission 585 nm filter.

CALCULATION OF RESULTS

1. Subtract blank OD (S4) from the standard OD values and plot the OD against standard concentrations. Determine the slope using linear regression fitting.
2. Conversions: 1 µg/mL glycerol equals 10.9 µM.
3. The glycerol concentration of Sample is calculated:

   Glycerol (µg/mL) = $(OD_{Sample}-OD_{Blank})$ / Slope (Colorimetric procedure)

   Glycerol (µg/mL) = $(RFU_{Sample} – RFU_{Blank})$ / Slope (Fluorimetric procedure)

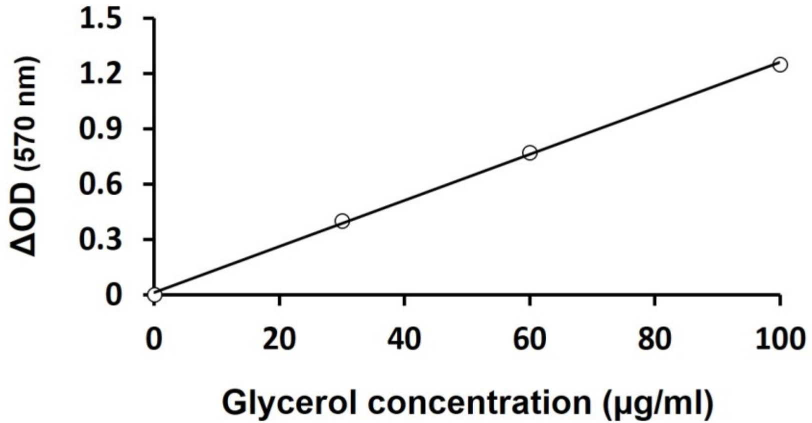
   Note:

   OD_{Sample} and OD_{Blank}: Optical density value of the sample and Blank (S4, medium only)

   RFU_{Sample} and RFU_{Blank}: Relative fluorescence unit of the sample and Blank (S4, medium only)

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Adipolysis (Lipolysis) Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.92 µg/mL