Adipolysis Assay Kit (Colorimetric/Fluorometric)

LS-K229-200 (200 Tests) • **Store at -20°C**

LSBio
LifeSpan BioSciences, Inc.

Introduction

Obesity is a chronic condition that develops from storage of excessive energy in the form of adipose tissue. The resulting adiposity presents a high risk factor for diseases such as type 2 diabetes, cardiovascular diseases, and cancer. ADIPOLYSIS or lipolysis is a highly regulated process in fat metabolism, in which triglycerides are broken down into glycerol and free fatty acids. Rapid, robust and accurate procedures for Adipolysis quantification in cell culture are very useful in research and drug discovery. LSBio's Adipolysis assay kit directly measures glycerol released during Adipolysis. This homogeneous assay uses a single Working Reagent that combine's glycerol kinase, glycerol phosphate oxidase and color reactions in one step. The color intensity of the reaction product at 570nm is directly proportional to glycerol concentration in the sample.

Key Features

- Sensitive and accurate. Use as little as 10 µL samples. Linear detection range in 96 -well plate: 0.92 to 100 µg/mL (10 to 1000 µM) glycerol for colorimetric assays and 0.2 to 5 µg/mL for fluorometric assays.
- Rapid and convenient. The procedure involves addition of a single working reagent and incubation for 20 min at room temperature.
- Robust and amenable to HTS assays. Potential interference by testing drugs is greatly reduced at 570nm. Compatible with culture media containing phenol red. Assay s can be performed in 96 or 384-well plates.

Applications

- Direct Assays: Adipolysis (glycerol in cell culture media).
- Drug Discovery/Pharmacology: effects of testing drugs on Adipolysis.

Components

Component	K229-200
	200 Tests
Assay Buffer	24 mL
Enzyme Mix	500 µL
ATP	250 µL
Dye Reagent	220 µL
Standard (100 mM Glycerol)	100 µL

Materials Not Supplied

Pipetting devices, centrifuge tubes, appropriate 96- or 384-well plates and plate reader.

Storage

The kit is shipped on ice. Store all kit components at -20 °C.

Adipolysis Assay Kit (Colorimetric/Fluorometric)

LS-K229-200 (200 Tests) • **Store at -20°C**

LSBio
LifeSpan BioSciences, Inc.

Assay Procedure

Colorimetric Procedure

SH-group containing reagents (e.g. mercaptoethanol, DTT) may interfere with this assay and should be avoided in sample preparation. Prior to the assay, equilibrate all components to room temperature. Keep thawed Enzyme Mix in a refrigerator or on ice during assays.

1. Cell Culture. Note: Cells and testing drugs are to be provided by the customer and are not included in this reagent kit. 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.
2. Standards and Samples. Prepare a 100 µg/mL standard by mixing 10 µL 100 mM glycerol standard with 910 µL in the same medium used for cell culture. Dilute standard in the medium as follows. Transfer 10 µL standards into wells of a clear 96-well assay plate (5 µL for 384-well assay plate).

No	100 µg/mL STD + Medium	Vol (µL)	Glycerol (µg/mL)
1	400 µL + 0 µL	400	100
2	300 µL + 200 µL	500	60
3	150 µL + 350 µL	500	30
4	0 µL + 500 µL	500	0

3. Collect cell culture supernatants from culture wells. Such samples should be assayed immediately or stored at -20°C. Transfer 10 µL samples (5 µL for 384-well assay plate) into separate wells of the assay plate.
4. Enzyme Reaction. For each assay well, mix 100 µL Assay Buffer, 2µL Enzyme Mix, 1 µL ATP and 1 µL Dye Reagent in a clean tube. Transfer 100 µL Working Reagent into each assay well. Tap plate to mix. For assays in a 384-well plate, use 50 µL Working Reagent per well.
5. Incubate 20 min at room temperature. Read optical density at 570nm (550-585nm).

   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.

Fluorometric Procedure

For fluorometric assays, the linear detection range is 0.2 to 5 µg/mL glycerol.

1. Dilute Standards (#1 to # 4, see Colorimetric Procedure) as follows: mix 10 µL standard with 190 µL dH2O. The glycerol concentrations are now 5.0, 3.0, 1.5 and 0 µg/mL, respectively.
2. Cell culture supernatant: dilute by mixing 10 µL cell culture sample with 190 µL dH2O (dilution factor n = 20).
3. Transfer 5 µL of the diluted standards and samples into separate wells of a black 96-well or 384-well plate.
4. Add 50 µL Working Reagent and tap plate to mix.
5. Incubate 20 min at room temperature and read fluorescence at λex = 530nm and λem = 585nm.

6. The glycerol concentration of Sample is calculated as

$$[\text{Glycerol}] = \frac{F_{SAMPLE} - F_{MEDIUM}}{\text{Slope}} \times 20 \quad (\mu g/mL)$$

Calculations

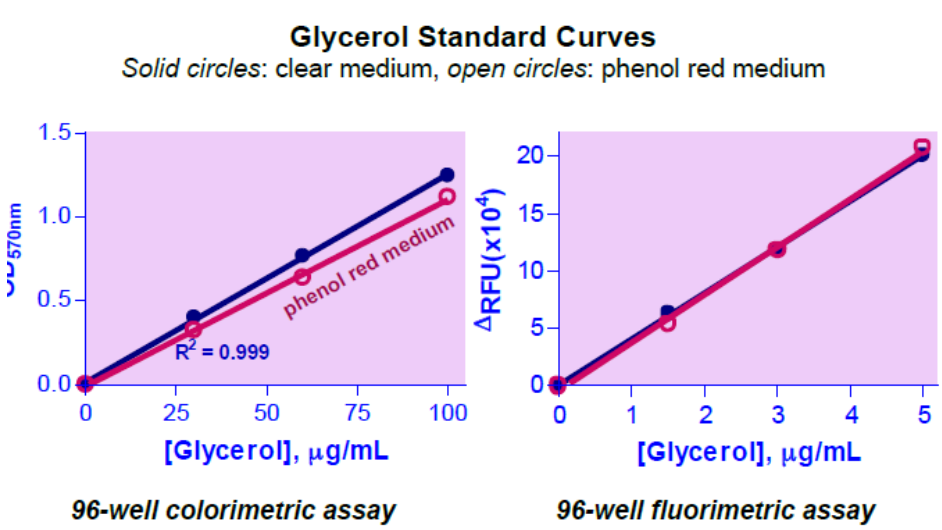
Subtract blank OD (#4) from the standard OD values and plot the OD against standard concentrations. Determine the slope using linear regression fitting. The glycerol concentration of Sample is calculated

$$[\text{Glycerol}] = \frac{OD_{SAMPLE} - OD_{MEDIUM}}{\text{Slope}} \quad (\mu g/mL)$$

OD_{SAMPLE} and OD_{MEDIUM} are optical density values of the sample and medium (#4). Conversions: 1 µg/mL glycerol equals 10.9 µM.

Sample Data

Pipetting devices, centrifuge tubes, appropriate 96- or 384-well plates and plate reader.



Version: V.08.09.2018