Technical Manual

ChromaDazzle Adipolysis Assay Kit

Catalogue Code: BA0076

Pack Size: 200 assays

Research Use Only

DESCRIPTION

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. The Assay Genie ChromaDazzle Adipolysis Assay Kit directly measures glycerol released during adipolysis. This homogeneous assay uses a single Working Reagent that combines 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 fluorimetric 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. Assays 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.

KIT CONTENTS (bulk reagents available)

Assay Buffer: 24 mL **Enzyme Mix:** 500 µL **ATP**: 250 µL

Dye Reagent: 220 µL **Standard**: 100 µL 100 mM Glycerol

Storage conditions. The kit is shipped on ice. Store Assay Buffer at 4°C and other reagents at -20°C. Shelf life of 12 months after receipt.

Precautions: 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.

COLORIEMTRIC 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

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.

3. *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.

4. 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.

CALCULATION
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.

FLUORIMETRIC PROCEDURE
For fluorimetric assays, the linear detection range is 0.2 to 5 µg/mL glycerol. Dilute Standards (#1 to # 4, see *Colorimetric Procedure*) as follows: mix 10 µL standard with 190 µL dH_2O. The glycerol concentrations are now 5.0, 3.0, 1.5 and 0 µg/mL, respectively.

Cell culture supernatant: dilute by mixing 10 µL cell culture sample with 190 µL dH_2O (dilution factor *n* = 20).

Transfer 5 µL of the diluted standards and samples into separate wells of a *black* 96-well or 384-well plate.

Add 50 µL Working Reagent and tap plate to mix.

Incubate 20 min at room temperature and read fluorescence at λ_{ex} = 530nm and λ_{em} = 585nm.

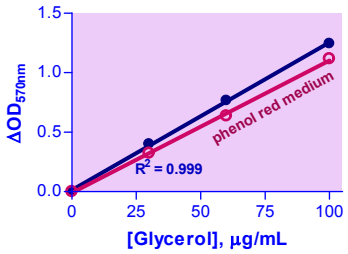
The glycerol concentration of Sample is calculated as

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

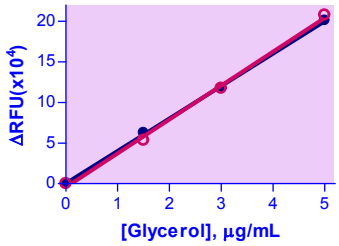
MATERIALS REQUIRED, BUT NOT PROVIDED
Pipeting devices, centrifuge tubes, appropriate 96- or 384-well plates and plate reader.

Glycerol Standard Curves

Solid circles: clear medium, *open circles*: phenol red medium



96-well colorimetric assay



96-well fluorimetric assay

LITERATURE

1. Duncan RE, et al. (2007). Regulation of lipolysis in adipocytes. Annu Rev Nutr. 27:79-101.

2. Moller F, Roomi MW. (1974). An enzymatic, spectrophotometric glycerol assay with increased basic sensitivity. Anal Biochem. 59(1):248-58.

3. MacRae AR. (1977). A semi-automated enzymatic assay for free glycerol and triglycerides in serum or plasma. Clin Biochem. 10(1):16-9.

Contact Details

Dublin, Ireland

Email: info@assaygenie.com

Web: www.assaygenie.com

Technical Support: Techsupport@assaygenie.com