

## EnzyChrom™ Adipolysis Assay Kit (Cat# EAPL-200)

### Quantitative Colorimetric/Fluorimetric Assay for Adipolysis

#### 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. BioAssay Systems' 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  $\mu$ L samples. Linear detection range in 96-well plate: 0.92 to 100  $\mu$ g/mL (10 to 1000  $\mu$ M) glycerol for colorimetric assays and 0.2 to 5  $\mu$ g/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  $\mu$ L **ATP:** 250  $\mu$ L

**Dye Reagent:** 220  $\mu$ L **Standard:** 100  $\mu$ L 100 mM Glycerol

**Storage conditions.** The kit is shipped on ice. Store all components 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.

#### 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  $\mu$ g/mL standard by mixing 10  $\mu$ L 100 mM glycerol standard with 910  $\mu$ L in the same medium used for cell culture. Dilute standard in the medium as follows. Transfer 10  $\mu$ L standards into wells of a clear 96-well assay plate (5  $\mu$ L for 384-well assay plate).

| No | 100 $\mu$ g/mL STD + Medium | Vol ( $\mu$ L) | Glycerol ( $\mu$ g/mL) |
|----|-----------------------------|----------------|------------------------|
| 1  | 400 $\mu$ L + 0 $\mu$ L     | 400            | 100                    |
| 2  | 300 $\mu$ L + 200 $\mu$ L   | 500            | 60                     |
| 3  | 150 $\mu$ L + 350 $\mu$ L   | 500            | 30                     |
| 4  | 0 $\mu$ L + 500 $\mu$ L     | 500            | 0                      |

Collect cell culture supernatants from culture wells. Such samples should be assayed immediately or stored at -20°C. Transfer 10  $\mu$ L samples (5  $\mu$ L for 384-well assay plate) into separate wells of the assay plate.

3. **Enzyme Reaction.** For each assay well, mix 100  $\mu$ L Assay Buffer, 2  $\mu$ L

Enzyme Mix, 1  $\mu$ L ATP and 1  $\mu$ L Dye Reagent in a clean tube. Transfer 100  $\mu$ L Working Reagent into each assay well. Tap plate to mix.

*For assays in a 384-well plate, use 50  $\mu$ L 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  $\mu$ g/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

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

OD<sub>SAMPLE</sub> and OD<sub>MEDIUM</sub> are optical density values of the sample and medium (#4). **Conversions:** 1  $\mu$ g/mL glycerol equals 10.9  $\mu$ M.

#### FLUORIMETRIC PROCEDURE

For fluorimetric assays, the linear detection range is 0.2 to 5  $\mu$ g/mL glycerol. Dilute Standards (#1 to # 4, see *Colorimetric Procedure*) as follows: mix 10  $\mu$ L standard with 190  $\mu$ L dH<sub>2</sub>O. The glycerol concentrations are now 5.0, 3.0, 1.5 and 0  $\mu$ g/mL, respectively.

Cell culture supernatant: dilute by mixing 10  $\mu$ L cell culture sample with 190  $\mu$ L dH<sub>2</sub>O (dilution factor  $n = 20$ ).

Transfer 5  $\mu$ L of the diluted standards and samples into separate wells of a black 96-well or 384-well plate.

Add 50  $\mu$ L Working Reagent and tap plate to mix.

Incubate 20 min at room temperature and read fluorescence at  $\lambda_{\text{ex}} = 530\text{nm}$  and  $\lambda_{\text{em}} = 585\text{nm}$ .

The glycerol concentration of Sample is calculated as

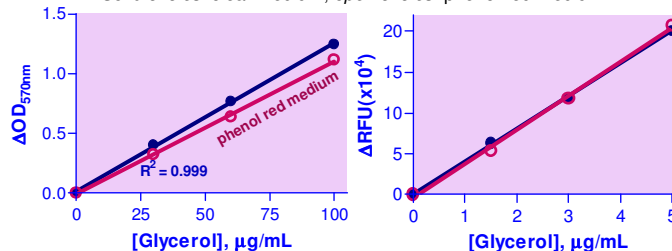
$$[\text{Glycerol}] = \frac{F_{\text{SAMPLE}} - F_{\text{MEDIUM}}}{\text{Slope}} \times 20 \quad (\mu\text{g/mL})$$

#### 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

#### PUBLICATIONS

- Jocken, Johan WE, et al (2018). Short-chain fatty acids differentially affect intracellular lipolysis in a human white adipocyte model. *Frontiers in endocrinology* 8: 372.
- Huttala, Outi, et al (2019). Presence of vasculature results in faster insulin response in adipocytes in vascularized adipose tissue model. *ALTEX-Alternatives to animal experimentation*.
- Nascimento, EBM et al (2021). Nicotinamide riboside enhances in vitro beta-adrenergic brown adipose tissue activity in humans. *The Journal of Clinical Endocrinology and Metabolism*.

