EnzyChrom™ Adipogenesis Assay Kit (Cat# EAPG-200)

Quantitative Colorimetric/Fluorimetric Adipogenesis Determination

DESCRIPTION

Adipogenesis is a tightly regulated cellular differentiation process, in which mesenchymal stem cells commit to preadipocytes and preadipocytes differentiate into adipocytes. Adipocytes, processing the largest energy reserve as triglycerol in the body of animals, play a key role in energy homeostasis. An increasingly sedentary lifestyle coupled with an energy-rich diet has contributed to a high frequency of obesity and other health problems, such as type 2 diabetes. Simple, direct and automation-ready procedures for measuring adipogenesis find wide applications in research and drug discovery. BioAssay Systems' EAPG-200 Assay Kit determines adipogenesis, in which triglycerides are extracted, hydrolyzed to glycerol and measured using a Dye Reagent. The color intensity at 570nm or fluorescence intensity at FL530/585nm is directly proportional to glycerol concentration in the sample.

KEY FEATURES

Sensitive and accurate. Use as little as 40 µL samples. Linear detection range in 96-well plate: 0.16 to 5 nmoles for colorimetric assays and 0.075 to 0.5 nmoles for fluorimetric assays.

Fast and convenient. The addition of a single working reagent and 30-min incubation procedure combines sample extraction, hydrolysis and color reaction. The whole procedure is performed at room temperature with *no incubator or heating* needed.

Robust and amenable to HTS. Homogeneous "mix-incubate-measure" type assay. No wash and reagent transfer steps are involved. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

APPLICATIONS:

For sensitive quantitative determination of adipogenesis and high-throughput screening of adipogenesis modulators.

KIT CONTENTS

Assay Buffer: 24 mL
Extraction Solution: 8 mL
ATP: 250 µL
Dye Reagent: 220 µL
Enzyme Mix: 500 µL
Lipase: 1000 µL
Standard: 100 µL 100 mM Glycerol

Storage conditions. The kit is shipped on ice. Store all components at -20°C. Shelf life of 6 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.

PROCEDURES

Note: (1) SDS and SH-group containing reagents (e.g. mercaptoethanol, DTT) may interfere with this assay and should be avoided in sample preparation; (2) Samples may contain free glycerol, a sample blank is recommended to correct for the background glycerol, by adding Blank Working Reagent (BWR) with *no Lipase*.

Equilibrate all components to room temperature. Keep thawed Lipase and Enzyme Mix in a refrigerator or on ice.

Colorimetric Assays:

1. Standard curve. Prepare 1 mM glycerol standard by mixing 10 µL 100mM glycerol standard and 990 µL dH_2O. Dilute to 0.125 mM by mixing 100 µL 1 mM and 700 µL dH_2O. Dilute Standards in dH_2O in wells of a clear 96-well plate.

No	STD + H_2O	Vol (µL)	Glycerol (nmol/well)
1	40 µL + 0 µL	40	5.0
2	24 µL + 16 µL	40	3.0
3	12 µL + 28 µL	40	1.5
4	0 µL + 40 µL	40	0

2. Sample preparation. Cells: 10 - 50 x 10^3 cells (e.g. preadipocytes, adipocytes) are mixed with 100 µL Extraction Solution, vortex for 30 seconds. Centrifuge samples for 5 min at 14,000 rpm. Transfer 40 uL cell lysate (corresponding to 4 – 20 x 10^3 cells) to sample wells. For each sample, transfer 40 µL cell lysate to a separate well as "Sample Blank".
   Tissue sample (e.g. 1-5 mg) is homogenized in 100 µL Extraction Solution. If tissue lysate is used, directly mix 1-10 µg Sample with 100 µL Extraction Solution, vortex for 30 seconds. Centrifuge samples for 5 min at 14,000 rpm. Dilute samples with dH_2O to 25-250 µg/mL. Transfer 40 µL Sample (1-10 µg) into separate wells.
3. Assay. Prepare Working Reagent (WR) for each *Standard and Sample* well, by mixing 60 µL Assay Buffer, 2 µL Enzyme Mix, 5 µL Lipase, 1 µL ATP and 1 µL Dye Reagent in a clean tube. Prepare Blank Working Reagent (BWR) for each *Sample Blank* well, by mixing 65 µL Assay Buffer, 2 µL Enzyme Mix, (*no Lipase*), 1 µL ATP and 1 µL Dye Reagent in another clean tube.
   Transfer 60 µL WR into standards and sample wells, 60 µL BWR into Sample Blank wells only. Tap plate to mix.
   Incubate 30 min at room temperature. Read optical density at 570nm (550-585nm).

Note: If the Sample OD is higher than the Standard OD at 5 nmoles glycerol, dilute sample in water and repeat the assay. Multiply by the dilution factor *n*.

Fluorimetric Assays: The Fluorimetric assay procedure is the same as the Colorimetric assay, except that it is more sensitive and uses a black flat-bottom 96-well plate. The glycerol standards will be 0.5, 0.3, 0.15 and 0 nmoles/well. Samples should be diluted at least 10-fold as for the Colorimetric assay procedure, i.e., 0.4 - 2 x 10^3 cells/well, or 0.1 – 1 ug tissue extract/well. Read fluorescence at λ_{ex} = 530 nm and λ_{em} = 585 nm.

CALCULATION

Subtract OD_{H2O} (water, #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 as:

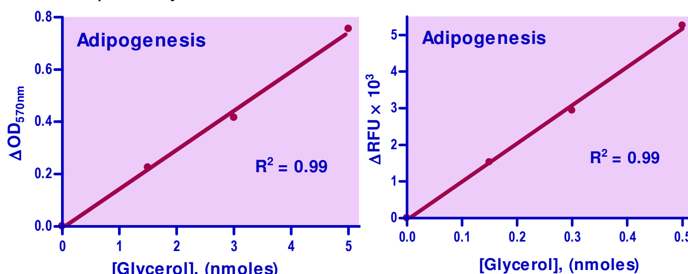
$$[\text{Glycerol}] = \frac{S_{SAMPLE} - S_{BLANK}}{\text{Slope}} \times n \quad \text{(nmoles)}$$

Where S_{SAMPLE} and S_{BLANK} are the OD or fluorescence intensity values of the Sample and Sample Blank wells. n is the dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices, centrifuge tubes, clear flat bottom 96-well plates (e.g. Corning Costar), black flat bottom 96-well plates, and plate reader.

Examples: HEPG2 cells, rat liver tissue and fish bone tissue were assayed using the 96-well procedure. The results are: 59.7 ± 5.0 nmoles/10^6 cells, 104.3 ± 5.8 nmoles/mg and 70.2 ± 0.7 nmoles/mg tissue, respectively.



Standard Curves. *Left*: Colorimetric assay; *Right*: Fluorimetric assay

LITERATURE

1. Moseti D, Regassa A, Kim WK (2016). Molecular Regulation of Adipogenesis and Potential Anti-Adipogenic Bioactive Molecules. Int J Mol Sci. 17(1):124.
2. Mota de Sá P, et al (2017). Transcriptional Regulation of Adipogenesis. Compr Physiol. 7(2):635-674.
3. Ghaben AL, Scherer PE (2019). Adipogenesis and metabolic health. Nat Rev Mol Cell Biol. 20(4):242-258.

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