Adipogenesis Assay Kit (BA0236)

Product Datasheet | SKU BA0236

Description

A quantitative colorimetric/fluorimetric assay for adipogenesis. Triglycerides are extracted, hydrolysed to glycerol and measured using a Dye Reagent; the colour intensity at 570 nm or fluorescence intensity at 530/585 nm is directly proportional to glycerol concentration in the sample.

Specifications

Detection Method	Colorimetric (OD570 nm, 550-585 nm) / Fluorimetric (λex/em = 530/585 nm)
Detection Range	Colorimetric: 0.16 to 5 nmoles; Fluorimetric: 0.075 to 0.5 nmoles per 96-well
Sample Type	Cells (e.g. preadipocytes, adipocytes) and tissue
Species Reactivity	All
Assay Time	30 minutes
Kit Size	200 Assays
Equipment Required	Microplate reader
Storage	The kit is shipped on ice. Store all components at -20°C.
Shelf Life	6 months after receipt.
Shipping	Gel Pack

Kit Components

Component	Quantity	Storage
Assay Buffer	24 mL	-20°C
Extraction Solution	8 mL	-20°C
ATP	250 µL	-20°C
Dye Reagent	220 µL	-20°C
Enzyme Mix	500 µL	-20°C
Lipase	1000 µL	-20°C
Standard (100 mM Glycerol)	100 µL	-20°C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0236	200 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. Equilibrate all components to room temperature; keep thawed Lipase and Enzyme Mix in a refrigerator or on ice. Avoid SDS and SH-group containing reagents (e.g. mercaptoethanol, DTT) in sample preparation, and include a Sample Blank to correct for background glycerol.
2. Colorimetric standard curve: prepare 1 mM glycerol standard by mixing 10 µL 100 mM glycerol standard and 990 µL dH2O; dilute to 0.125 mM by mixing 100 µL 1 mM and 700 µL dH2O. Dilute standards in dH2O in wells of a clear 96-well plate (5.0, 3.0, 1.5 and 0 nmol/well per the table).
3. Sample preparation: cells (10-50 × 10^3 preadipocytes/adipocytes) are mixed with 100 µL Extraction Solution, vortexed 30 s and centrifuged 5 min at 14,000 rpm; transfer 40 µL cell lysate to sample wells

and 40 µL to a separate Sample Blank well. Tissue (1-5 mg) is homogenised in 100 µL Extraction Solution, or 1-10 µg tissue lysate is mixed with 100 µL Extraction Solution, vortexed and centrifuged; dilute to 25-250 µg/mL and transfer 40 µL Sample per well.

4. Prepare Working Reagent (WR) per Standard and Sample well: 60 µL Assay Buffer, 2 µL Enzyme Mix, 5 µL Lipase, 1 µL ATP and 1 µL Dye Reagent. Prepare Blank Working Reagent (BWR) per Sample Blank well: 65 µL Assay Buffer, 2 µL Enzyme Mix (no Lipase), 1 µL ATP and 1 µL Dye Reagent.
5. Transfer 60 µL WR into standards and sample wells and 60 µL BWR into Sample Blank wells only. Tap plate to mix. Incubate 30 min at room temperature. Read optical density at 570 nm (550-585 nm).
6. Fluorimetric assay: as above but more sensitive, using a black flat-bottom 96-well plate; glycerol standards 0.5, 0.3, 0.15 and 0 nmoles/well. Dilute samples at least 10-fold. Read fluorescence at λex = 530 nm and λem = 585 nm.

Colorimetric Standard Curve

No.	STD	H2O	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

Calculation

Subtract OD_H2O (water, #4) from the standard OD values and plot OD against standard concentrations to determine the Slope by linear regression. [Glycerol] = [(S_Sample – S_Blank) / Slope] × n (nmoles), where S_Sample and S_Blank are the OD or fluorescence intensity values of the Sample and Sample Blank wells and n is the dilution factor. If the Sample OD is higher than the Standard OD at 5 nmoles glycerol, dilute sample in water and repeat the assay, multiplying by n.

Notes

- SDS and SH-group containing reagents (e.g. mercaptoethanol, DTT) may interfere with this assay and should be avoided in sample preparation.
- 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.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.