SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Adipolysis Assay Kit
for cell culture supernatants

Catalog Number **MAK313**
Storage Temperature -20°C

TECHNICAL BULLETIN

Product 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.

This adipolysis assay kit directly measures glycerol released during adipolysis in cultured cells. This homogeneous assay uses a single Reaction Mix that combines glycerol kinase, glycerol phosphate oxidase, and color reactions in one step, resulting in a colorimetric (570 nm)/fluorimetric (λ_{ex} = 530/ λ_{em} = 587 nm) product, proportional to the glycerol present.

Sensitive and accurate – Use cell culture samples as small as 10 μL. Linear detection range in 96 well plate: 0.92–100 μg/mL (10–1,000 μM) glycerol for colorimetric assays and 0.2–5 μg/mL for fluorimetric assays.

Rapid and convenient – The procedure involves addition of a single working reagent and incubation for 20 minutes at room temperature.

Robust and amenable to HTS assays – Potential interference by test compounds in the cell culture medium is greatly reduced at 570 nm. Compatible with culture media containing phenol red. Assays can be performed in 96 or 384 well plates.

Components

The kit is sufficient for 200 assays in 96 well plates.

Component	Amount
Assay Buffer Catalog Number MAK313A	24 mL
Dye Reagent Catalog Number MAK313B	220 μL
Enzyme Mix Catalog Number MAK313C	500 μL
ATP Catalog Number MAK313D	250 μL
Standard, 100 mM Glycerol Catalog Number MAK313E	100 μL

Reagents and Equipment Required but Not Provided.

- 96-well flat-bottom plate. It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays. Cell culture or tissue culture treated plates are **not** recommended.
- Spectrophotometric or fluorescence multiwell plate reader
- Cells to be cultured and test compounds

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

The Assay Buffer may contain a precipitate after storage at –20 °C. This precipitate does not impact the performance of the reagent. Equilibrating the solution at room temperature for several hours or warming in a water bath from 37 °C to 60 °C should dissolve most of the precipitate. Allow the buffer to return to room temperature before beginning the assay.

Storage/Stability
The kit is shipped on dry ice. Storage at –20 °C is recommended. The Assay Buffer can be stored at 2–8 °C after thawing.

Procedure
All samples and standards should be run in duplicate. Use ultrapure water for the preparation of fluorimetric standards and samples.

Colorimetric Procedure
Note: SH-group containing reagents (e.g., 2-mercapto-ethanol, 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 – Grow cells (e.g., preadipocytes, adipocytes) in culture plate (24, 96, or 384-well). If desired, treat cells with test compounds such as insulin or isoproterenol, and incubate for the desired time period.
   Note: Cells and test compounds are to be provided by the end user and are not included in this reagent kit.

2. Standards and Samples – Prepare a 100 μg/mL glycerol standard by mixing 10 μL of the 100 mM glycerol standard with 910 μL of the same medium used to culture the cells. Dilute standard in the medium as shown in Table 1. Transfer 10 μL of standards into wells of a clear 96-well assay plate (5 μL for 384-well assay plate).

Table 1.
Preparation of Standards

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.

2. Reaction Mix - Mix enough reagents for the number of assays to be performed. For each well, prepare 100 μL of Reaction Mix according to Table 2. Note: For assays in a 384 well plate, use 50 μL of Reaction Mix per well.

   Table 2.
   Preparation of Reaction Mix

Reagent	Volume
Assay Buffer	100 μL
Enzyme Mix	2 μL
ATP	1 μL
Dye Reagent	1 μL

3. Enzyme Reaction – Transfer 100 μL of Reaction Mix into each assay well. Tap plate to mix.
   Note: For assays in a 384-well plate, use 50 μL of Reaction Mix per well.

4. Incubate 20 minutes at room temperature. Read optical density at 570 nm (550–585nm).
   Note: if the Sample OD is higher than the Standard OD at 100 μg/mL, dilute sample with ultrapure water and repeat the assay. Multiply result by the dilution factor.

Results

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 0.2–5 μg/mL of glycerol.

Dilute Standards (#1 to # 4, see Colorimetric Procedure) as follows: Mix 10 μL of 100 μg/mL glycerol standard with 190 μL of ultrapure water. The glycerol concentrations are now 5.0, 3.0, 1.5, and 0 μg/mL, respectively.

Cell culture supernatant – Dilute by mixing 10 μL of cell culture supernatant with 190 μL of ultrapure water (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 Reaction Mix and tap plate to mix.

Incubate 20 minutes at room temperature and read fluorescence (λ_{ex} = 530/λ_{em} = 587 nm)

The glycerol concentration of Sample is calculated as

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

References

1. Duncan, R.E. et al., Regulation of lipolysis in adipocytes. *Annu. Rev. Nutr.*, **27**, 79-101 (2007).
2. Moller, F., and Roomi, M.W., An enzymatic, spectrophotometric glycerol assay with increased basic sensitivity. *Anal. Biochem.*, **59**(1), 248-58 (1974).
3. MacRae, A.R., A semi-automated enzymatic assay for free glycerol and triglycerides in serum or plasma. *Clin. Biochem.*, **10**(1), 16-9 (1977).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96-well plate used	For fluorescence assays – black plates with clear bottoms; for colorimetric assays – clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

SS,KVG,SJ,JAC,MAM 11/20-1

©2020 Sigma-Aldrich Co. LLC. All rights reserved. SIGMA-ALDRICH is a trademark of Sigma-Aldrich Co. LLC, registered i the US and other countries. Sigma brand products are sold through Sigma-Aldrich, Inc. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see product information on the Sigma-Aldrich website at www.sigmaaldrich.com and/or on the reverse side of the invoice or packing slip.