SIGMA-ALDRICH®

Product Information

Lipolysis (3T3-L1) Colorimetric Assay Kit

Catalog Number **MAK211**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
Lipolysis is the process of hydrolyzing triglycerides to free fatty acids and glycerol. This process involves the action of adipose TG lipase (ATGL), hormone-sensitive lipase (HSL), and monoglyceride lipase. Lipolysis maintains the energy balance during fasting and exercise by providing a substrate for oxidative metabolism. Lipolysis is regulated by nutritional factors and hormones. Problems with the regulation of lipolysis is associated with obesity, diabetes, and metabolic syndromes.[1,2]

The Lipolysis (3T3-L1) Colorimetric Assay Kit contains a synthetic catecholamine (isoproterenol) that activates β-adrenergic receptors. This activates adenylate cyclase, converting ATP to cAMP. cAMP then activates the hydrolysis of triglycerides by hormone-sensitive lipase. Lipolysis is determined by measuring a colorimetric product with absorbance at 570 nm (A_{570}) proportional to the amount of glycerol present.

Components
The kit is sufficient for 100 assays in 96 well plates.

Lipolysis Assay Buffer Catalog Number MAK211A	17 mL
Lipolysis Wash Buffer Catalog Number MAK211B	22 mL
Glycerol Assay Buffer Catalog Number MAK211C	25 mL
Glycerol Probe, in DMSO Catalog Number MAK211D	0.2 mL
Glycerol Enzyme Mix Catalog Number MAK211E	1 vl
Glycerol Standard, 100 mM Catalog Number MAK211F	0.2 mL
Isoproterenol, 10 mM Catalog Number MAK211G	50 μL

Reagents and Equipment Required but Not Provided
- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader
- Microscope

Precautions and Disclaimer
This product is 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. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Lipolysis Assay Buffer and Lipolysis Wash Buffer –
    Store at 2–8 °C or –20 °C. Warm to 37 °C before use.

Glycerol Assay Buffer – Store at –20 °C. Warm to room temperature before use.

Glycerol Probe – Store at –20 °C. Warm to 37 °C for 1–2 minutes to melt DMSO before use. Mix well by pipetting. Use within 2 months.

Glycerol Enzyme Mix – Reconstitute with 220 μL of Glycerol Assay Buffer. Completely dissolve and mix by gentle pipetting. Aliquot and store at –20 °C. Keep on ice while in use. Use within 2 months.

Glycerol Standard, 100 mM – Ready to use. Store at –20 °C.

Isoproterenol, 10 mM – Dilute 1 μL of 10 mM Isoprotenerol Stock Solution in 999 μL of water to make a 10 μM Isoproterenol Working Solution. Store at –20 °C. Warm to room temperature before use. Use within 2 months.

Storage/Stability
The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Glycerol Standards
Dilute 10 µL of the 100 mM Glycerol standard with 990 µL of Glycerol Assay Buffer and mix well to make a 1 mM (1 nmole/µL) Glycerol Standard Solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM (1 nmole/µL) Glycerol Standard Solution into a 96 well plate generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Glycerol Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Grow and differentiate 3T3-L1 adipocytes in a 96 well plate until oil droplets are visible with a microscope. Wash the cells twice with 100 µL of Lipolysis Wash Buffer. Discard the wash buffer. Add 150 µL of Lipolysis Assay Buffer. Use caution when washing to avoid detaching cells.

Stimulation of lipolysis: Add 1.5 µL of 10 µM isoproterenol (final concentration of 100 nM) to the sample cells in which lipolysis stimulation is required. Stimulate lipolysis by incubating the plate for 1–3 hours or longer.

Transfer 20–50 µL of the medium to a fresh 96 well plate. Bring samples to a final volume of 50 µL of Lipolysis Assay Buffer.

Notes: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

High concentrations of isoproterenol interfere with detection. For samples that require greater than 100 nM isoproterenol to stimulate lipolysis, it is recommended to spike the standards also with the same concentration of isoproterenol.

For samples exhibiting significant background, include a Sample Blank for each sample by omitting the Glycerol Enzyme Mix. The Sample Blank readings can then be subtracted from the sample readings.

Assay Reaction
1. Set up the appropriate Reaction Mixes according to the scheme in Table 1. 50 µL of Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	Standards and samples	Sample Blank
Glycerol Assay Buffer	46 µL	48 µL
Glycerol Enzyme Mix	2 µL	–
Glycerol Probe	2 µL	2 µL

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting.

3. Incubate the plate for 30 minutes at room temperature. Protect plate from light during incubation.

4. Measure the absorbance (A_{570}) in a microplate reader. The signals are stable for 2 hours.

Results

Calculations

Correct for the background by subtracting the measurement obtained for the 0 (blank) Glycerol Standard from that of the standards and samples. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Glycerol Standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the Sample Blank value from the Sample value to obtain the corrected measurement. Using the corrected measurement, determine the amount of glycerol (nmole/well) generated by the assay (S_a).

Concentration of Glycerol

$$C = S_a/S_v$$

where:
S_a = Amount of glycerol in unknown sample well (nmole) from standard curve
S_v = Sample volume (μL) added into the well
C = Concentration of glycerol in sample (nmole/μL)

Sample Calculation

Amount of Glycerol (S_a) = 5.84 nmole (from standard curve)

Sample volume (S_v) = 20 μL

Concentration of glycerol in sample:

$$5.84 \text{ nmole}/20 \text{ } \mu L = 0.292 \text{ nmole}/\mu L$$

Molecular weight of glycerol: 92.09 g/mole

$$0.292 \text{ nmole}/\mu L \times 92.09 \text{ ng/nmole} = 26.9 \text{ ng}/\mu L$$

References

1. Ghorbani, A., and Abedinzade, M., Comparison of *in vitro* and *in situ* methods for studying lipolysis. ISRN Endocrinol., 205385 (2013) doi: 10.1155/2013/205385 (2013).
2. Nielsen, T.S. et al., Dissecting adipose tissue lipolysis: molecular regulation and implications for metabolic disease. J. Mol. Endocrinol., **52**, R199–222 (2014).

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 colorimetric assays, use 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 needed to use 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 Mixes before each 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 Mixes
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare Reaction Mixes 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

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