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Product Information

Lipolysis (Adipocyte) Kit

Catalog Number **MAK195**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

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

The Lipolysis (Adipocyte) Kit provides reagents for adipocyte isolation from rat or mouse adipose tissue, induction of lipolysis, stimulation of the cAMP-mediated pathway by synthetic catecholamine (isoproterenol), and measurement of glycerol released. The kit can detect <200 pmole and <20 pmole of glycerol by colorimetric and fluorometric assay, respectively. It may also be used to screen compounds that affect lipolysis and to study metabolic dysfunctions.

Components

The kit is sufficient for 5 g of tissue.

Collagenase Catalog Number MAK195A	10 mL
Collagenase Stop Buffer Catalog Number MAK195B	90 mL
Adipocyte Wash Buffer Catalog Number MAK195C	22 mL
Adipocyte Lipolysis Buffer Catalog Number MAK195D	17 mL
Glycerol Assay Buffer Catalog Number MAK195E	25 mL
Glycerol Probe Catalog Number MAK195F	0.2 mL
Glycerol Enzyme Mix Catalog Number MAK195G	1 vl
Glycerol Standard Catalog Number MAK195H	0.2 mL
Isoproterenol Catalog Number MAK195I	50 µL
Cell Strainer Catalog Number MAK195J	1 EA

Reagents and Equipment Required but Not Provided

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays and black plates for fluorometric assays.
- Spectrophotometric or fluorometric multiwell plate reader
- 50 mL conical tubes
- Orbital shaker
- Dissecting scissors

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. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Collagenase (0.2%) – Aliquot and store at –20 °C; thaw on ice and during use. Use within 2 months.

Collagenase Stop Buffer – Store at 2–8 °C or –20 °C; warm to 37 °C before use.

Adipocyte Wash Buffer – Store at 2–8 °C or –20 °C; warm to 37 °C before use.

Adipocyte Lipolysis 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 – 20 °C; warm to 37 °C for 1–2 minutes and mix well to dissolve before use. Use within 2 months.

Glycerol Enzyme Mix – Store at 2–8 °C or –20 °C; warm to 37 °C before use.

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 for Colorimetric Detection
Prepare 1 mM Glycerol Standard by adding 10 μL of the Glycerol standard to 990 μL Adipocyte Lipolysis Buffer. Add 0, 2, 4, 6, 8, and 10 μL of the 1 mM standard solution into a 96 well plate generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Adipocyte Lipolysis Buffer to each well to bring the volume to 50 μL.

Glycerol Standards for Fluorometric Detection
Prepare 20 μM Glycerol Standard by adding 20 μL of the 1 mM Glycerol standard to 980 μL Adipocyte Lipolysis Buffer. Add 0, 2, 4, 6, 8, and 10 μL of the 20 μM standard solution into a 96 well plate generating 0 (blank), 40, 80, 120, 160, and 200 pmole/well standards. Add Adipocyte Lipolysis Buffer to each well to bring the volume to 50 μL.

Sample Preparation
Isolation of adipocytes – Mince freshly isolated adipose tissue thoroughly for 5 minutes using dissecting scissors. Place the minced tissue in a 50 mL conical tube and add 0.2% Collagenase (2 mL/1 g of tissue). Incubate in an orbital shaker at 37 °C for 30 minutes at 160 rpm with cap loosened. Add Collagenase Stop Buffer (9 mL/1 mL of 0.2% Collagenase solution), mix by inverting, and filter through 100 μm cell strainer. Centrifuge the filtrate at 500 × *g* for 10 minutes and transfer the top layer (adipocytes) into a fresh tube.
Note: Mince the tissue thoroughly with scissors for proper digestion. In most cases, digestion of tissue with Collagenase for 20–45 minutes is sufficient.

Stimulation of lipolysis: Wash adipocytes twice with Adipocyte Wash Buffer (2 mL/1 g tissue). Centrifuge at 500 × *g* for 10 minutes. Discard the wash buffer and add Adipocyte Lipolysis Buffer (1.5 mL/1 g tissue). Make 150 μL aliquots of adipocytes in separate tubes for each reaction. Add 1.5 μL of 10 μM Isoproterenol (final concentration of 100 nM) to the samples in which lipolysis stimulation is required. Stimulate lipolysis by incubating the tubes at 37 °C for 1–3 hours or longer.

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.

A higher concentration of Isoproterenol (≥5 μM) interferes with detection. For samples that require higher concentrations of Isoproterenol to stimulate lipolysis, it is recommended to spike the standards also with the same concentration of Isoproterenol.

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

Table 1.
Reaction Mixes

Reagent	Colorimetric Assay	Fluorometric Assay
Glycerol Assay Buffer	46 μL	47.6 μL
Glycerol Probe	2 μL	0.4 μL*****
Glycerol Enzyme Mix	2 μL	2 μL

***** For fluorometric assay, use 0.4 μL of the probe/well to reduce fluorescence background

2. Add 50 μL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting. Incubate the plate at room temperature for 30 minutes, protected from light.
3. Measure absorbance (A_{570}) or fluorescence (λ_{ex} = 535/λ_{em} = 587 nm) in a microplate reader. The signals are stable for 2 hours.

Results

Calculations

Subtract the measurement (A_{570} or FLU) obtained for the blank from that of the standards and samples. Plot the standard curve. Apply the corrected reading of the sample to the standard curve to get B (nmole of glycerol) in the sample wells.

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

Glycerol Content of Tissue

Using the corrected measurement, determine the amount of glycerol present in the tissue (C):

$$C = B \times T/S \times D = \text{nmol/100 mg tissue}$$

where:

B = the amount of glycerol from standard curve (nmole)

T = the total volume of sample (150 μL)

S = volume of sample added into reaction well (μL)

D = the dilution factor

Molecular weight of glycerol: 92.09 g/mole

Glycerol content may be expressed as nmole or nmole/100 mg tissue; alternatively as nmole/μg of protein or nmole/μg of lipid.

References

1. Ghorbani, A., and Adedinzade, M., ISRN Endocrinol, doi: 10.1155/2013/205385 (2013).
2. Nielsen, T.S., J. Mol. Endocrinol., **52**, R199 (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 Master Reaction Mix 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 mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master 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

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