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Product Information

Adipogenesis Assay Kit

Catalog Number **MAK040**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Synonym: Triglyceride Assay Kit

Product Description

Adipogenesis is the differentiation of pre-adipocytes derived from pluripotent mesenchymal cells into mature adipocytes. Adipocytes, which are capable of storing large quantities of lipids as triglycerides, are the main cells for lipid storage in animals. Adipose tissue plays a critical role in the regulation of whole-body energy homeostasis and adipocyte dysfunction has been implicated in pathological states such as cancer, type II diabetes, cardiovascular disease, and atherosclerosis.

In this kit, total cellular concentrations of triglycerides are determined by a coupled enzyme assay, which results in a colorimetric (570 nm)/ fluorometric (λ_{Ex} = 535/λ_{Em} = 587 nm) product, proportional to the triglycerides present. This kit is able to detect triglycerides in as few as 1×10^4 differentiated 3T3 L1 cells.

Components

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

Component	Amount
Adipogenesis Assay Buffer Catalog Number MAK040A	25 mL
Lipid Extraction Solution Catalog Number MAK040B	10 mL
Adipogenesis Probe in DMSO Catalog Number MAK040C	0.2 mL
Lipase Catalog Number MAK040D	1 vial
Adipogenesis Enzyme Mix Catalog Number MAK040E	1 vial
Triglyceride Standard, 1 mM Catalog Number MAK040F	0.3 mL

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.
- Fluorescence or spectrophotometric multiwell plate reader
- 3T3 L1 Cell Line (Catalog Number 86052701), optional

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.

Adipogenesis Assay Buffer – Allow buffer to come to room temperature before use.

Adipogenesis Probe – Warm to room temperature to thaw the solution prior to use. Store protected from light and moisture at –20 °C.

Lipase and Adipogenesis Enzyme Mix – Reconstitute with 220 μL of Adipogenesis Assay Buffer. Mix well by pipetting (don't vortex), then aliquot and store, protected from light and moisture, at –20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

Triglyceride Standard – Standard may separate during storage. To reconstitute, thaw tube and then place in a hot water bath (80–100 °C) for 1 minute until substrate looks cloudy. Vortex for 30 seconds. Repeat heat and vortex one more time. The substrate should look clear. The substrate is now ready for use. Aliquot and store remainder at –20 °C.

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.

Triglyceride Standards for Colorimetric Detection

Dilute 50 μL of the 1 mM Triglyceride Standard with 200 μl of the Adipogenesis Assay Buffer to prepare a 0.2 mM standard solution. Add 0, 10, 20, 30, 40, and 50 μL of the 0.2 mM standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Adipogenesis Assay Buffer to each well to bring the volume to 50 μL.

Triglyceride Standards for Fluorometric Detection

Prepare a 0.2 mM solution as for the colorimetric assay. Dilute 40 μL of the 0.2 mM solution with 360 μL of the Adipogenesis Assay Buffer to prepare a 20 μM solution. Add 0, 10, 20, 30, 40, and 50 μL of the 20 μM standard solution into a 96 well plate, generating 0 (blank), 0.2, 0.4, 0.6, 0.8, and 1 nmole/well standards. Add Adipogenesis Assay Buffer to each well to bring the volume to 50 μL.

Sample Preparation

Adipocyte precursors, such as, 3T3 cells, human preadipocytes cells cultured in a multiwell plate: Remove medium from cell wells and wash once with room temperature PBS. Add 100 μL of Lipid Extraction Buffer per well, seal plate, and incubate for 30 minutes at 90–100 °C. Solution should be cloudy. Cool plate to room temperature and shake plate for 1 minute to homogenize solution.

> Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve. Fully differentiated 3T3-L1 cells can contain greater than 100 times the triglycerides of uninduced cells.

Bring samples to a final volume of 50 μL with Adipogenesis Assay Buffer.

Assay Reaction

1. Add 2 μL of Lipase solution to each sample and standard well and incubate for 10 minutes at room temperature to convert triglycerides to glycerol and fatty acids.

2. Set up the Master Reaction Mix according to the scheme in Table 1 (colorimetric) or Table 2 (fluorometric). 50 μL of the Master Reaction Mix is required for each reaction (well).

> Note: Detection sensitivity is 10-100 fold higher for the fluorometric assay. For the fluorometric assay, the Adipogenesis Probe must be diluted 1:10 with Adipogenesis Assay Buffer prior to use to decrease the background readings, therefore increasing detection sensitivity.

Table 1.

Colorimetric Master Reaction Mix

Reagent	Volume
Adipogenesis Assay Buffer	46 μL
Adipogenesis Probe	2 μL
Adipogenesis Enzyme Mix	2 μL

Table 2.

Fluorometric Master Reaction Mix

Reagent	Volume
Adipogenesis Assay Buffer	46 μL
Adipogenesis Probe (diluted 1:10)	2 μL
Adipogenesis Enzyme Mix	2 μL

3. Add 50 μL of the appropriate Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting and incubate the reaction for 30 minutes at 37 °C. Protect the plate from light during the incubation.

4. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{Ex} = 535/λ_{Em} = 587 nm).

Results

Calculations

The background for either assay is the value obtained for the 0 (blank) triglyceride standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate triglyceride standards to plot a standard curve. The amount of triglyceride present in the samples may be determined from the standard curve.

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

Concentration of Triglyceride

$S_a/S_v = C$

S_a = Amount of Triglyceride in unknown sample (nmole) from standard curve

S_v = Sample volume (μL) added to reaction well

C = Concentration of triglyceride in sample

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, use black plates with clear bottoms. 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 improperly stored reagents	Check the storage requirements 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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