ab287843 – 3T3-L1 Differentiation Kit

For in vitro differentiation of 3T3-L1 preadipocytes to adipocytes
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab287843

Introduction

3T3-L1 cells are derived from mouse 3T3 cells and provide a widely-used fundamental model in the study of adipose physiology and metabolic diseases. They exhibit a fibroblast-like morphology before differentiation but become rounded and accumulate lipid droplets several days after the initiation of differentiation. The accumulated lipid droplets can be visualized by light microscopy. The *in vitro* differentiated 3T3-L1 adipocytes result in characteristics similar to tissue-derived adipocytes and have been commonly used to study adipogenesis, lipolysis, and metabolic dysfunctions. This 3T3-L1 Differentiation Kit provides enough supplements to make 100 ml of differentiation medium and 600 ml of maintenance medium which is sufficient material for 12 100 mm culture dishes. The Differentiation Cocktail provides a final concentration of 1.5 µg/ml insulin, 1 µM dexamethasone, 500 µM IBMX, and 1 µM rosiglitazone in the differentiation media.

Applications

- Differentiation of 3T3-L1 preadipocytes to adipocytes
- Study of obesity, adipogenesis, lipolysis and lipid metabolism

Sample Type

- Animal tissues: primary preadipocytes
- Cell culture: 3T3-L1 cells

Storage and Stability

An unopened kit can be stored at -20°C for 6 months.

Materials Supplied

Item	Quantity	Storage Condition
Insulin	600 µl	-20°C
1000X Differentiation Cocktail	1 vial	-20°C
DMSO I	0.5 ml	-20°C

PLEASE NOTE: DMSO I was previously labelled as DMSO (anhydrous). The composition has not changed.

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Cells grown in 96-well, 6-well, or 100 mm cell culture plate
- DMEM, DMEM/F12 (1:1), bovine calf serum, fetal bovine serum (FBS)
- Penicillin, streptomycin
- 0.22 µM syringe filters
- Light microscope

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Insulin: Ready to use as supplied. Warm to room temperature before use. Aliquot the spare and store at -20°C. Avoid repeated freeze/thaw cycles. Stable for 6 months.

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1000X Differentiation Cocktail: Reconstitute in 110 µl DMSO I (supplied), making sure the material is completely dissolved. Aliquot the spare and store at -20°C. Avoid repeated freeze/thaw. Stable for 6 months.

Cell Culture

- Culture 3T3-L1 (ATCC® CL-173) in preadipocyte medium consisting of DMEM media with 10% bovine calf serum, 100 units/ml penicillin and 100 µg/ml streptomycin in a humidified incubator at 37°C with 5% CO_2.

Δ Notes:

a) Important: Do not allow cultures to become confluent until initiation of differentiation to avoid overgrown culture. Change medium every 2-3 days and routinely split prior to initiating differentiation.
b) It is important to subculture preadipocytes in a medium with 10% bovine calf serum.

Assay Protocol

Differentiation Induction:

1. To initiate differentiation, culture cells until ~80% confluent.
2. Replace medium with fresh preadipocyte medium and incubate an additional 48 hrs.
3. Add 1 µl of Differentiation Cocktail to 1 ml of DMEM/F12 (1:1) with 10% FBS. Make enough differentiation medium as needed. Sterilize with a 0.22 µM syringe filter. Replace preadipocyte medium with differentiation medium.
4. Incubate for 3 days in a humidified incubator at 37°C with 5% CO_2.

Δ Notes:

a) It may be necessary to screen several lots of FBS, as some may be better at differentiation than others.
b) Primary preadipocytes may differentiate better at 10% CO_2.

Maintenance:

1. Prepare maintenance medium by adding 1 µl of Insulin to 1 ml of DMEM/F12 (1:1) with 10% FBS. Filter sterilize with 0.22 µM syringe filter.
2. Remove differentiation medium and replace with maintenance medium.
3. Replace medium every 2-3 days.

- Lipid droplet accumulation will be visible by light microscopy 7-10 days after the addition of differentiation medium.

Δ Note: *Enough maintenance medium can be prepared for several medium changes. Store the unused maintenance medium at 4°C.*

Technical Support

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