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Product Information

3T3-L1 Differentiation Kit

Catalog Number **DIF001**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

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 have characteristics similar to tissue-derived adipocytes and have been commonly used to study adipogenesis, lipolysis, and metabolic dysfunctions.

The 3T3-L1 Differentiation Kit provides enough supplements to make:

100 ml of Differentiation Medium
600 ml of Maintenance Medium

This is sufficient material for twelve 100 mm culture dishes.

Components

Insulin (1.5 mg/ml) (Catalog Number DIF001A, Green Cap)	0.6 ml
Differentiation Cocktail, 1000× (lyophilized) (Catalog Number DIF001B, Yellow Cap)	1 vl
DMSO (anhydrous) (Catalog Number DIF001C, Blue Cap)	0.5 ml

Reagents and Equipment Required but Not Provided.

- Cells grown in 96 well, 6 well, or 100 mm cell culture plate
- DMEM and DMEM/F12 (1:1) media
- Bovine calf serum and fetal bovine serum (FBS)
- Penicillin and streptomycin
- 0.2 μm syringe filters
- Light 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. Read the entire protocol before preparing reagents.

Insulin – Supplied 1.5 mg/ml solution is ready to use. Warm to room temperature before use. Aliquot and store at –20 °C. Use within 6 months.

Differentiation Cocktail – Reconstitute in 110 μl of DMSO (supplied), making sure the material is completely dissolved. Aliquot and store at –20 °C. Use within 6 months.

Preadipocyte Medium – DMEM medium with 10% bovine calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin.

Differentiation Medium – Add 1 μl of Differentiation Cocktail to 1 ml of DMEM/F12 (1:1) medium with 10% FBS. Filter Sterilize with a 0.2 μm syringe filter. Make enough differentiation medium as needed. 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 Medium.

Maintenance Medium – Add 1 μl of Insulin to 1 ml of DMEM/F12 (1:1) with 10% FBS. Filter sterilize with 0.2 μm syringe filter. Enough Maintenance Medium can be prepared for several medium changes. Store the unused maintenance medium at 2–8 °C.

Storage/Stability

Store the kit at –20 °C, protected from light.

Procedure

3T3-L1 Preadipocyte Differentiation Protocol

In vitro differentiated 3T3-L1 adipocytes have characteristics similar to tissue-derived adipocytes and have been commonly used to study adipogenesis, lipolysis, and metabolic dysfunctions.

Either primary preadipocytes from animal tissues or cultured 3T3-L1 cells may be used in this procedure.

1. Culture 3T3-L1 cells in Preadipocyte Medium in a humidified incubator at 37 °C with 5% CO_2.
   Notes: It is important to never allow cultures to become confluent until initiation of differentiation. Change medium every 2–3 days.

   It is important to subculture preadipocytes in a medium with 10% bovine calf serum.

2. To initiate differentiation, culture cells until confluent. Replace medium with fresh Preadipocyte Medium and incubate an additional 48 hours.

3. Replace Preadipocyte Medium with Differentiation Medium. Incubate for 3 days in a humidified incubator at 37 °C with 5% CO_2.
   Notes: It may be necessary to screen several lots of FBS, as some may be better at differentiation than others.

   Primary preadipocytes may differentiate better at 10% CO_2.

4. Remove Differentiation Medium and replace with Maintenance Medium. Replace Maintenance Medium every 2–3 days. Lipid droplet accumulation will be visible by light microscopy 7–10 days after the addition of Differentiation Medium.

Results

Figure 1.
3T3-L1 cells before differentiation

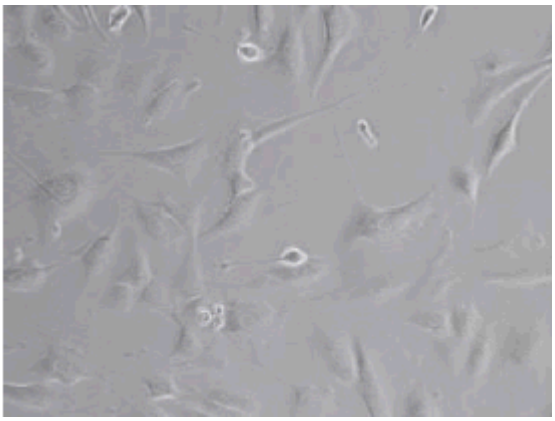
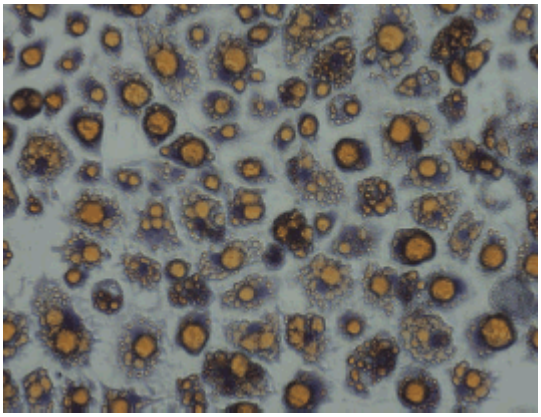


Figure 2.
3T3-L1 cells 7 days after the initiation of differentiation



Cells stained with neutral lipid dye, Oil Red O.

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