3T3-L1 Differentiation Kit

Simple, rapid & convenient method to differentiate 3T3-L1 preadipocytes to adipocytes

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K579
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 result in characteristics similar to tissue-derived adipocytes and have been commonly used to study adipogenesis, lipolysis, and metabolic dysfunctions. PromoCell's *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 x 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.
Quantity	1 Mix for 100 ml differentiation medium
Applications	• Differentiation of 3T3-L1 preadipocytes to adipocytes • Study of obesity, adipogenesis, lipolysis and lipid metabolism
Sample Type	• Cell culture: 3T3-L1 cells • Animal tissue: Primary preadipocytes (for primary human preadipocytes see Cat.Nos. C-12730 to C-12733)

Components	**Quantity**	**Color Code**
Insulin (1.5 mg/ml)	0.6 ml	Green Blue
Differentiation Cocktail, 1000x (lyophilized)	1 vial	Yellow
DMSO (anhydrous)	0.5 ml	Blue

User Supplied Reagents & Equipment	• 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
Storage and Reagents Preparation	Upon arrival, store kit at –20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay. • **Insulin**: Ready to use as supplied. Warm to room temperature before use. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Stable for 6 months. • **Differentiation Cocktail**: Reconstitute in 110 µl DMSO (supplied), making sure the material is completely dissolved. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Stable for 6 months.
Assay Protocol	**1. Cell Culture**: Culture 3T3-L1 (ATCC® CL-173TM) 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. Notes: a. Important: Never allow cultures to become confluent until initiation of differentiation. Change medium every 2-3 days. b. It is Important to subculture preadipocytes in a medium with 10% bovine calf serum. **2. Differentiation Induction**: To initiate differentiation, culture cells until confluent. Replace medium with fresh preadipocyte medium and incubate an additional 48 hours. Add 1 µl of Differentiation Cocktail to 1 ml of DMEM/F12 (1:1) with 10% FBS. Make as much differentiation medium as needed. Sterilize with a 0.22 µM syringe filter. Replace preadipocyte medium with differentiation medium. Incubate for 3 days in a humidified incubator at 37°C with 5% CO_2.

	Note: 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. **3. Maintenance**: 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. Remove differentiation medium and replace with maintenance medium. Replace medium every 2-3 days. Lipid droplet accumulation will be visible by light microscopy 7-10 days after the addition of differentiation medium. Note: Enough maintenance medium can be prepared for several medium changes. Store the unused maintenance medium at 4°C.

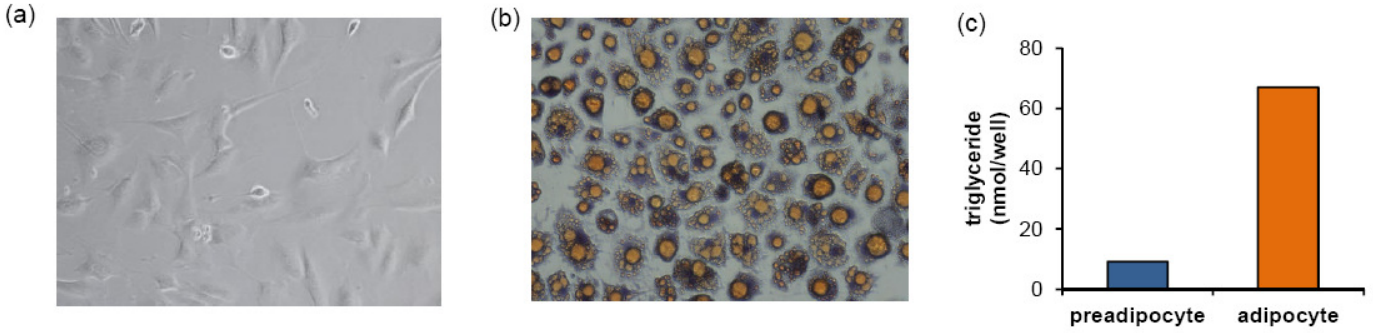

Figure: (a) 3T3-L1 cells before differentiation. (b) 3T3-L1 cells 7 days after the initiation of differentiation and stained with neutral lipid dye Oil Red O (Lipid Staining Kit; Cat.No. PK-CA577-K580). (c) Triglyceride levels in preadipocytes grown in a 96-well cell culture plate and in adipocytes 7 days after differentiation.

PromoCell GmbH

Sickingenstr. 63/65
69126 Heidelberg
Germany

Email: info@promokine.info
www.promokine.info

USA/Canada
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 90 93 32 (ligne verte)
Téléfax: 0800 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 – 96 03 33 (toll free)
Fax: 0800 – 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40

12/2017