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

Storage and Stability

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

Materials Supplied

Item	Quantity	Storage Condition
Insulin (1.5 mg/ml)	0.6 ml	-20°C
Differentiation Cocktail 1000x (Lyophilized)	1 vial	-20°C
DMSO (anhydrous)	0.5 ml	-20°C

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.

Differentiation Cocktail: Reconstitute in 110 µl DMSO (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% CO2.

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% CO2.

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% CO2.

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

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)