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BioVision
BioVision Incorporated

3T3-L1 Differentiation Kit

12/13

(Catalog # K579-100; for 100 ml differentiation medium; Store at -20°C)

I. **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. BioVision'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 12 100 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.

II. **Application:**

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

III. **Sample Type:**

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

IV. **Kit Contents:**

Components	K579-100	Cap Code	Part Number
Insulin (1.5 mg/ml)	0.6 ml	Green	K579-100-1
Differentiation Cocktail 1000x (Lyophilized)	1 vial	Yellow	K579-100-2
DMSO (anhydrous)	0.5 ml	Blue	K579-100-3

V. **User Supplied Samples, Reagents and 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

VI. **Storage and Handling:**

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

VII. **Reagent Preparation and Storage Conditions:**

- **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.

VIII. **3T3-L1 Preadipocyte Differentiation Protocol:**

1. **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.

   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 hrs. 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. Incubate for 3 days in a humidified incubator at 37°C with 5% CO_2.

   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.

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:

   a. Enough maintenance medium can be prepared for several medium changes. Store the unused maintenance medium at 4°C.

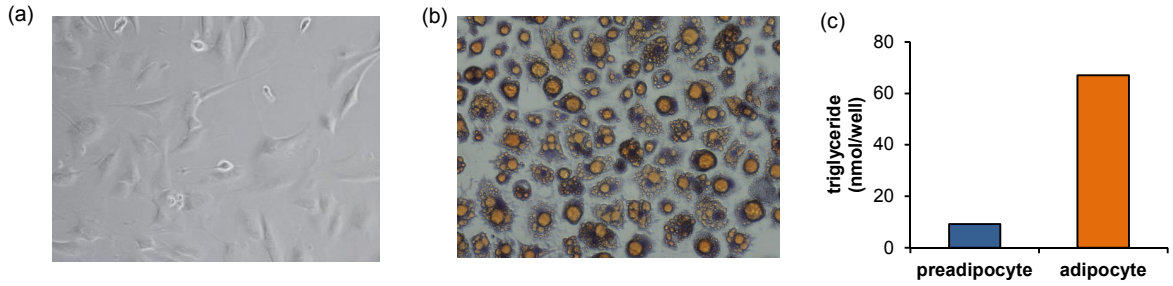

Figure: (a) 3T3-L1 cells before differentiation. (b) 3T3-L1 cells 7 days after the initiation of differentiation and stained with neutral lipid dye Oil Red O (Oil Red O staining kit [Cat # K580]). (c) Triglyceride levels in preadipocytes grown in a 96-well cell culture plate and in adipocytes 7 days after differentiation.

IX. **RELATED PRODUCTS:**

3T3-L1 Lipolysis Colorimetric Assay Kit (K577)
Insulin (human) ELISA Kit (K4742)
Adiponectin (human) Elisa Assay Kit (K4901)
Adiponectin (rat) Elisa Assay Kit (K4903)
Leptin (human) ELISA Kit (K4777)
Oil Red O Staining Kit (K580)
Preadipocyte Isolation Kit (K583)
3T3-L1 Lipolysis Fluorometric Assay Kit (K578)
Adipogenesis Colorimetric/Fluorometric Kit (K610)
Adiponectin (mouse) Elisa Assay Kit (K4902)
Resistin (human/mouse/rat) EIA Kit (K4767)
Glucose Uptake Assay Kits (K666, K676)
Glucose Colorimetric/Fluorometric Assay Kit (K606)
Triglyceride Quantification Colorimetric/Fluorometric Assay Kit (K622)

FOR RESEARCH USE ONLY! Not to be used on humans.