

CHEMICALLY-INDUCED DIFFERENTIATION OF ATCC® CL-173™ (3T3-L1) USING SINGLE-COMPONENT COMMERCIALY-AVAILABLE REAGENTS

INTRODUCTION

ATCC® CL-173™ (3T3-L1) materials from at least two different distribution lots were tested for their ability to differentiate in response to a mixture of insulin, dexamethasone, and IBMX (methylisobutylxanthine). The assay was established according to published protocols and is relatively easy to perform. The components listed here represent reagents that can be obtained individually from commercial suppliers.

SUPPLIES

- 3T3-L1 Mouse Embryonic Fibroblasts, ATCC® CL-173™
- Dulbecco's Modified Eagle's Medium (DMEM), ATCC® 30-2002
- Bovine Calf Serum, ATCC® 30-2030
- Fetal Bovine Serum (FBS), ATCC® 30-2020
- Insulin (bovine), Sigma® I0516
- Dexamethasone, G Biosciences® API-04
- Methylisobutylxanthine (IBMX), Sigma® I5879

MEDIA FORMULATIONS USED THROUGHOUT

Pre-adipocyte Expansion Medium:

90% Dulbecco's Modified Eagle's Medium (DMEM)
10% Bovine Calf Serum

Differentiation Medium:

90% Dulbecco's Modified Eagle's Medium (DMEM)
10% Fetal Bovine Serum (FBS)
1.0 µM Dexamethasone
0.5 mM Methylisobutylxanthine (IBMX)
1.0 µg/mL Insulin

Adipocyte Maintenance Medium:

90% Dulbecco's Modified Eagle's Medium (DMEM)
10% Fetal Bovine Serum
1.0 µg/mL Insulin

Pre-adipocyte Expansion Procedure:

Maintain the cells in the *Pre-adipocyte Expansion Medium* according to instructions for handling provided in the ATCC® CL-173™ product sheet. Seed the cells at about 3000 cells per cm², and never allow the cells to become confluent. Subculture 3T3-L1 cells before cultures reach 70% confluence (Figure 1).

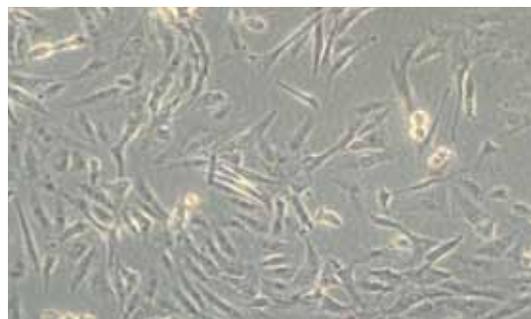


Figure 1. Light microscopy of subconfluent 3T3-L1 cells.

Differentiation Procedure:

1. When the cells are 70-80% confluent, harvest the cells by trypsinization according to the instructions related to subculture provided in the Product Sheet for ATCC® CL-173™.
2. Seed the cells in the desired culture vessel in the *Pre-adipocyte Expansion Medium*.

Culture Vessel	Cell Density
6 well plate	8 x10 ⁴ cells/well
24 well plate	2 x10 ⁴ cells/well
96 well plate	2 x10 ³ cells/wel

3. Let the cells grow for 48 hours, or until the culture reaches 100% confluence (Figures 2 & 3). Feed the cells with the *Pre-adipocyte Expansion Medium* after 48 hours.

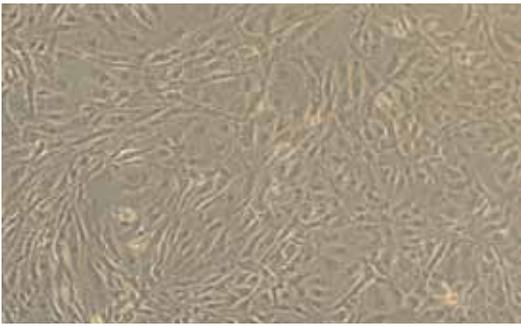


Figure 2. Light microscopy of confluent 3T3-L1 cells.

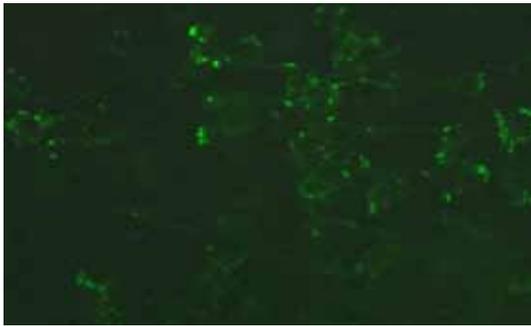


Figure 3. Confluent 3T3-L1 cells stained with Nile Red prior to induction.

4. Incubate the cells as a confluent culture for another 48 hours.
5. Remove the growth medium from each well and add an identical volume of *Differentiation Medium*.
6. Incubate in *Differentiation Medium* for 48 hours.
7. After 48 hours, replace the *Differentiation Medium* with *Adipocyte Maintenance Medium*.

NOTE: At this stage, the cells detach easily from the tissue culture vessel. Gentle pipetting/handling is recommended from this point forward.

8. Replace the *Adipocyte Maintenance Medium* every 48 to 72 hours.
9. The cells should be fully differentiated between 7 to 15 days after induction, as evidenced by observation of lipid droplet formation (Figures 4 & 5).

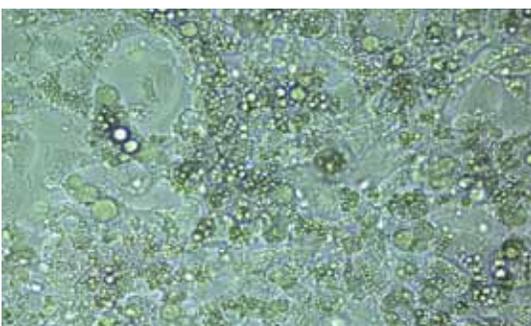


Figure 4. Light microscopy of differentiated 3T3-L1 cells.

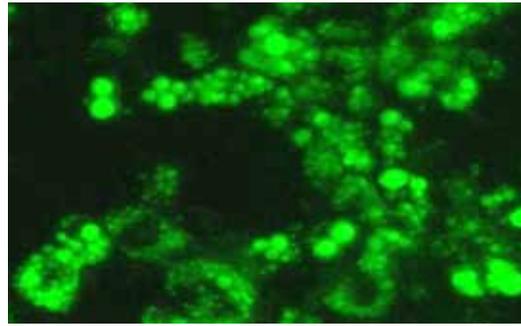


Figure 5. Differentiated 3T3-L1 cells stained with Nile Red.

TROUBLESHOOTING

Why do the cells peel off the substrate when I add the *Differentiation Medium*?

Possible Cause	Solution
pH and osmolality of the <i>Differentiation Medium</i> fall outside of the normal physiological range.	Check pH and osmolality of the <i>Differentiation Medium</i> prior to use. pH should fall between 7.0 and 7.4, and osmolality should fall within 270 to 365 mOsm/kg.
Cells were left for too long without a liquid layer.	Take great care to immediately add <i>Differentiation Medium</i> to the well once the <i>Pre-adipocyte Expansion Medium</i> is removed.

Why do the cells detach after I add the *Adipocyte Maintenance Medium*?

Possible Cause	Solution
The differentiated cells were handled too harshly and detached.	The cells easily detach once differentiation occurs. Take care when adding or removing medium. If the cells detach, add fresh medium to spent cultures and incubate for 48 hours.
Cells were left for too long without a liquid layer. pH and osmolality of the <i>Adipocyte Maintenance Medium</i> fall outside of the normal physiological range.	Check pH and osmolality of the <i>Adipocyte Maintenance Medium</i> prior to use. pH should fall between 7.0 and 7.4, and osmolality should fall within 270 to 365 mOsm/kg.
Cells were left for too long without a liquid layer.	Take great care to immediately add <i>Adipocyte Maintenance Medium</i> to the well once the <i>Differentiation Medium</i> is removed.

Why haven't the cells formed lipid droplets after 2 weeks?

Possible Cause	Solution
The wrong reagents were used (e.g. human insulin was substituted for bovine insulin).	If using an alternative supplier, be sure to check the quality, purity and/or characterization (e.g. cell-culture-tested) of the material prior to use.
The differentiation reagents were added to the medium in the wrong amounts.	Over- or under-supplementing the <i>Differentiation Medium</i> could keep the cells from forming lipid droplets; it may also, in the case of over-supplementing, cause irreversible harm to the cells.
The cells were not confluent prior to the addition of the <i>Differentiation Medium</i> .	Be sure to allow the culture to reach 100% confluence prior to changing to the <i>Differentiation Medium</i> (i.e. at confluence, the cells are already primed to differentiate).
The cells may not have been exposed to the <i>Differentiation Medium</i> long enough.	Once the <i>Differentiation Medium</i> is added, be sure to expose the cells for a full 48 hours.
Passage effect.	The cells may have been over-passaged or kept too dense during routine subculture.

REFERENCES

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