Differentiation of 3T3-L1 cells into adipocyte-like cells

This protocol outlines how to chemically induce the differentiation of 3T3-L1 cells into adipocyte-like cells.

3T3-L1 differentiation is an economical and convenient way to generate adipocyte-like cells for experiments.

Preparation of media

Preparation of media must be carried out in a tissue culture hood under aseptic conditions. MDI (methylisobutylxanthine, dexamethasone, insulin) induction medium and insulin medium should be freshly prepared.

Preparation of MDI induction medium:

- Prepare stock solutions of IBMX (50 mM) and dexathasone (1 mM) in DMSO. If using lyophilized insulin, reconstitute this according to the manufacturer's instructions.
- To DMEM, add IBMX to a final concentration of 0.5 mM (1 mL IBMX stock solution per 100 mL medium)
- Add dexamethasone to a final concentration of 1 µM (100 µL dexamethasone stock solution per 100 mL medium)
- Add insulin to a final concentration of 10 µg/mL

Preparation of insulin medium:

- To DMEM, add insulin to a final concentration of 10 µg/mL

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1. Seed cells in a six-well plate at a density of $3x10^3$ cells per cm^2.
2. Grow cells in DMEM until a confluency of 70% is reached, changing the medium every 2–3 days. Confluency should not exceed 70% before differentiation as this increases cell death after differentiation.
3. To initiate differentiation, remove DMEM and add 2–3 mL MDI induction medium per well **(Day 0)**.
4. On **Day 3:** Remove MDI induction medium from the cells and replace with 2–3 mL insulin medium.
5. On **Day 6:** Remove insulin medium from the cells and add fresh DMEM.
6. On **Day 7–10:** Fully differentiated adipocyte-like cells should be obtained.

Differentiation into adipocyte-like cells can be tracked by Oil Red O staining to monitor lipid accumulation, or by monitoring the expression of adipocyte markers such as adiponectin and FABP4.

Protocol adapted from Reed BC, Lane V (1980). Insulin receptor synthesis and turnover in differentiating 3T3-L1 preadipocytes. *Proc. Natl. Acad. Sci. USA.* 77, 285-289