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iXCells Protocol

ADIPOCYTE DIFFERENTIATION PROTOCOL

Adipocyte Differentiation Protocol is designed to achieve maximum adipocytes differentiation from adipose-derived stem cells (ADSCs) in vitro. This protocol can be used for human or rodent ADSCs.

Adipocyte Differentiation (12 well plate format)

1. Grow ADSCs in Adipose-Derived Stem Cell Growth Medium (Cat# MD-0003) to >95% confluency.
2. Aspirate the growth medium and replace with 1.5 ml fresh growth medium/well, let the cells grow for 2~3 more days.
3. Aspirate the growth medium, apply 1.5 ml Adipocyte Differentiation Medium (Cat# MD-0005) per well to the cells.

   Note: Cells at this stage may detach from dish easily, so do not use pump to aspirate off the medium at this step. Use pipet and slowly remove the medium instead. Add Adipocyte Differentiation Medium very gently to avoid cell detachment.
4. Change fresh Adipocytes Differentiation Medium every 3 days (slowly remove and add the medium as described above).
5. Culture human ADSCs in Adipocytes Differentiation Medium for 10-14 days, and analyze the percentage of cells with oil-droplet formation by Oil Red O Staining (Figure 1). Oil-droplet can be observed in 7-10 days post adipogenic induction when using rodent ADSCs (Figure 2).

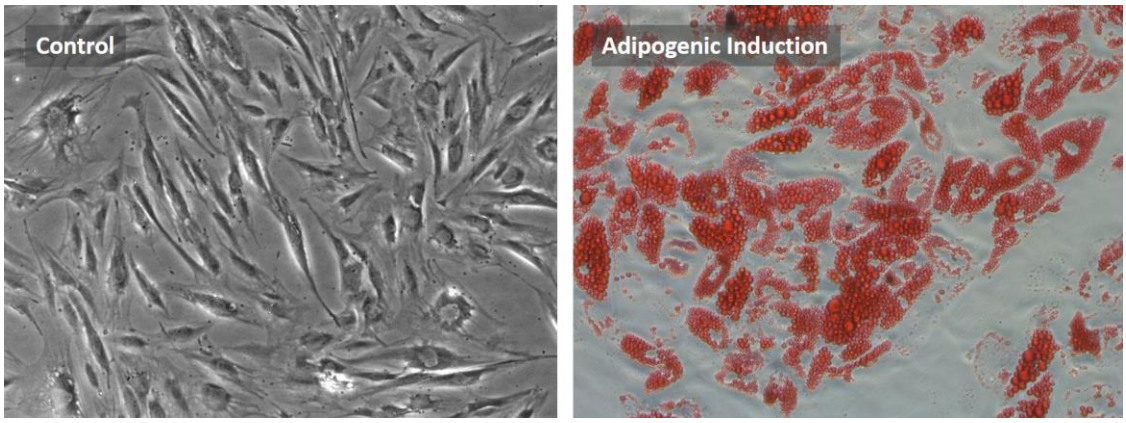

Figure 1. Human ADSC adipocyte differentiation (Day 14).

Oil Red O staining of adipocytes (12 well plate format)

1. Prepare Oil Red O Stock Solution.
   a. **Oil Red O Stock Solution:** Dissolve 0.35 g Oil Red O (Sigma, Cat# O-0625) in 100 ml of isopropanol. Stir overnight, filter (0.2 µM) and store at room temperature.
   b. **Oil Red O Working Solution:** Mix 6 ml of Oil Red O Stock Solution with 4 ml of ddH_2O. Let sit at room temperature for 20 minutes followed by filtering (0.2 µM). Oil Red O Working Solution need to be made freshly and immediately used for staining.
2. Remove the Adipocytes Differentiation Medium (Cat# MD-0005). Wash the cells once with 1.5 ml 1 x PBS.

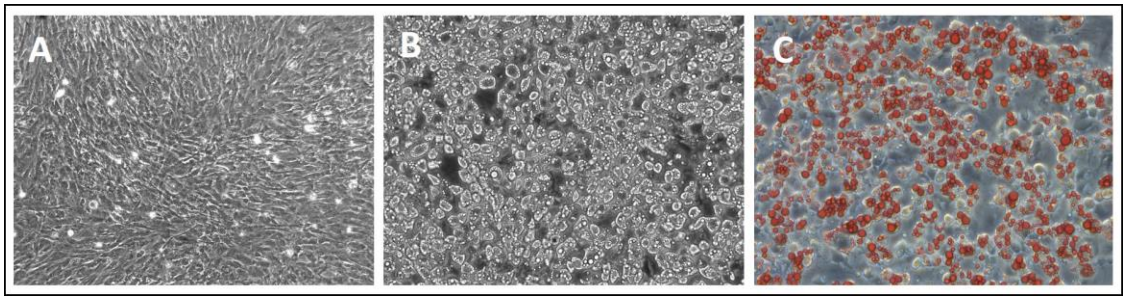

Figure 2. (A) Mouse ADSCs from white fat (phase contrast). (B) Adipocyte induction (Day 10, phase contrast). (C) Adipocyte induction (Day 10, Oil Red O staining).

3. Remove PBS. Apply 1 ml 10% formalin to the cells and incubate for 1 hour at room temperature.
4. Remove formalin with a pipette. Wash the cells twice with 1.5 ml ddH_2O. Wash cells with 1 ml of 60% isopropanol for 5 minutes at room temperature.
5. Let the cells air dry completely at room temperature for 8 minutes. Add 1 ml of freshly made Oil Red O Working Solution and incubate at room temperature for 10-30 minutes.
6. Remove Oil Red O Working Solution, and immediately wash the cells 2-4 times with 1.5 ml ddH_2O. Aspirate the wash solution and add 1 ml PBS/well.
7. Acquire images under the microscope for analysis.

Related Primary Cells:

Primary Cells	Vendor	Catalog #
Human Adipose Derived Stem Cells (hADSC, Normal)	iXCells Biotechnologies	10HU-001
Mouse Adipose-Derived Stem Cells-white fat (MADSC-wf)	iXCells Biotechnologies	10HU-006
Mouse Adipose-Derived Stem Cells-brown fat (MADSC-bf)	iXCells Biotechnologies	10MU-005
Rat Adipose Derived Stem Cells (rADSCs, from white fat)	iXCells Biotechnologies	10RA-001
Rat Adipose Derived Stem Cells (rADSCs, from brown fat)	iXCells Biotechnologies	10RA-002

Reagents/Media needed:

Reagent / Medium	Vendor	Catalog #
Adipose-Derived Stem Cell Growth Medium	iXCells Biotechnologies	MD-0003
Adipocyte Differentiation Medium	iXCells Biotechnologies	MD-0005
Oil Red O	Sigma	O-0625

Disclaimers