

## ADIPOCYTE DIFFERENTIATION PROTOCOL

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

### Adipocyte Differentiation (12 well plate format)

1. Grow preadipocytes in Preadipocyte Growth Medium (Cat# MD-0004) or 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 or preadipocytes 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 or preadipocytes (Figure 2).

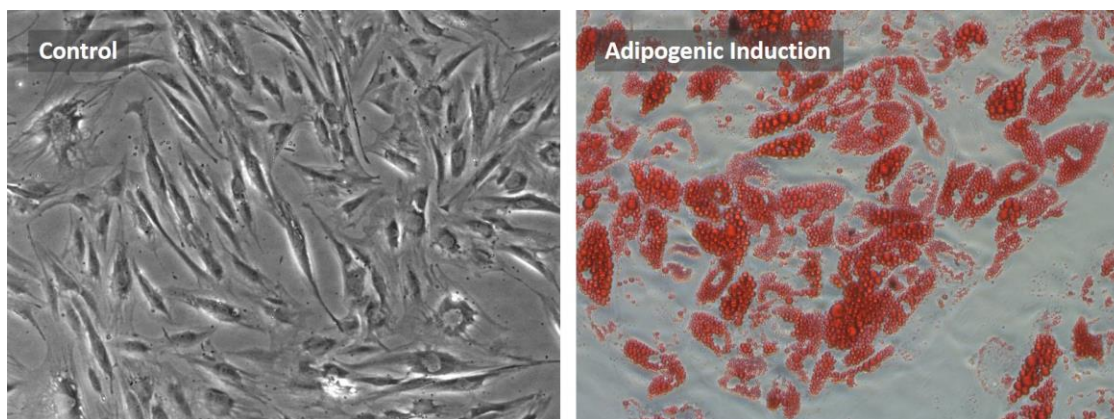
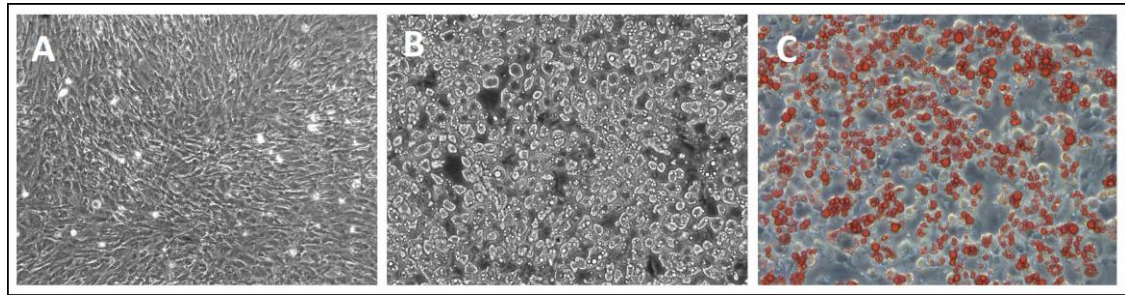


Figure 1. Human ADSC adipocyte differentiation (Day 14).

### 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 100 %isopropanol. Place on the stir plate for minimum of 1hr. Filter (0.4  $\mu$ M or smaller) and store at room temperature.  
**Note:** Notice the precipitate might cause the solution hard to filter.
  - b. **Oil Red O Working Solution:** Mix 6 ml of Oil Red O Stock Solution with 4 ml of ddH<sub>2</sub>O. Let sit at room temperature for 20 minutes followed by filtering (0.2  $\mu$ M). Oil Red O Working Solution need to be made freshly and immediately used for staining.



**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).

2. Remove the Adipocytes Differentiation Medium (Cat# MD-0005). Wash the cells once with 1.5 ml 1 x PBS.  
Note: The process of removing and adding should be every gentle.
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<sub>2</sub>O. Wash cells with 1 ml of 60% isopropanol for 5 minutes at room temperature.
5. Allow cells to dry at room temperature. Add 1 ml of freshly made Oil Red O Working Solution and incubate at room temperature for 10-30 minutes. or, until the red dye are coated.
6. Remove Oil Red O Working Solution, and immediately wash the cells 2 times with 1.5 ml ddH<sub>2</sub>O. 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
Human Preadipocytes / Adipocytes (hPADs)	iXCells Biotechnologies	10HU-101
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
Mouse Preadipocytes (mPADs, from white fat)	iXCells Biotechnologies	10MU-106
Mouse Preadipocytes (mPADs, from brown fat)	iXCells Biotechnologies	10MU-105
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
Preadipocyte Growth Medium	iXCells Biotechnologies	MD-0004

Adipocyte Differentiation Medium	iXCells Biotechnologies	MD-0005
Oil Red O	Sigma	O-0625

## Disclaimers

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