iXCells Biotechnologies USA, Inc.
7270 Trade ST, Suite 102
San Diego, CA 92121
www.ixcellsbiotech.com
Tel: 858-412-5988
Email: supports@ixcellsbiotech.com

iXCells Protocol

OSTEOGENIC DIFFERENTIATION PROTOCOL

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

Osteogenic differentiation from adipose-derived stem cells (ADSCs) (12 well plate)

1. Grow ADSCs in Adipose-Derived Stem Cell Growth Medium (Cat# MD-0003) to ~80% confluency.
2. Carefully aspirate the growth medium, apply 1.5 ml Osteogenic Differentiation Medium per well (Cat# MD-0006) to the cells.
3. Change fresh Osteogenic Differentiation Medium every 3 days. Be careful not to disturb the cell monolayer.
4. Culture the human ADSCs for 3-4 weeks, and osteoblasts can be detected by Alizarin Red S staining (stain the extracellular calcium deposit) (Figure 1 and 2). Extracellular calcium deposit can be observed in 2-3 weeks post osteogenic induction when using mouse ADSCs.

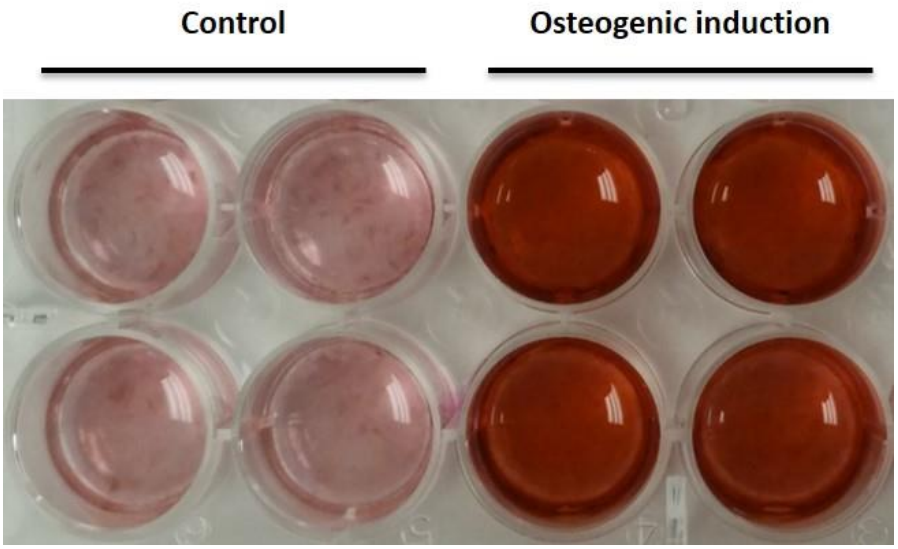

Figure 1. Human ADSC osteogenic induction (Day 24). Alizarin Red S staining of osteoblasts. The extracellular calcium deposit was stained in bright orange-red color.

Alizarin Red S staining of osteoblasts (12 well plate)

1. **Alizarin Red S Solution preparation:** Dissolve 2 g Alizarin Red S (Sigma, Cat# A5533) in 100 ml of ddH_2O and adjust pH to 4.1-4.3 with HCl or NH_4OH. Filter the solution through 0.22 µ membrane and store at 4°C in dark.
2. Remove the Osteogenic Differentiation Medium. Wash cells once with 1.5 ml 1 x DPBS (w/o Ca^{++}/Mg^{++}).
3. Apply 1 ml 10% formalin to the cells and incubate for 1 hour at room temperature.
4. Remove formalin with a pipette. Wash cells with 1.5 ml of ddH_2O twice.
5. Add 1 ml of freshly made Alizarin Red S Solution and incubate at room temperature in the dark for >45 minutes.

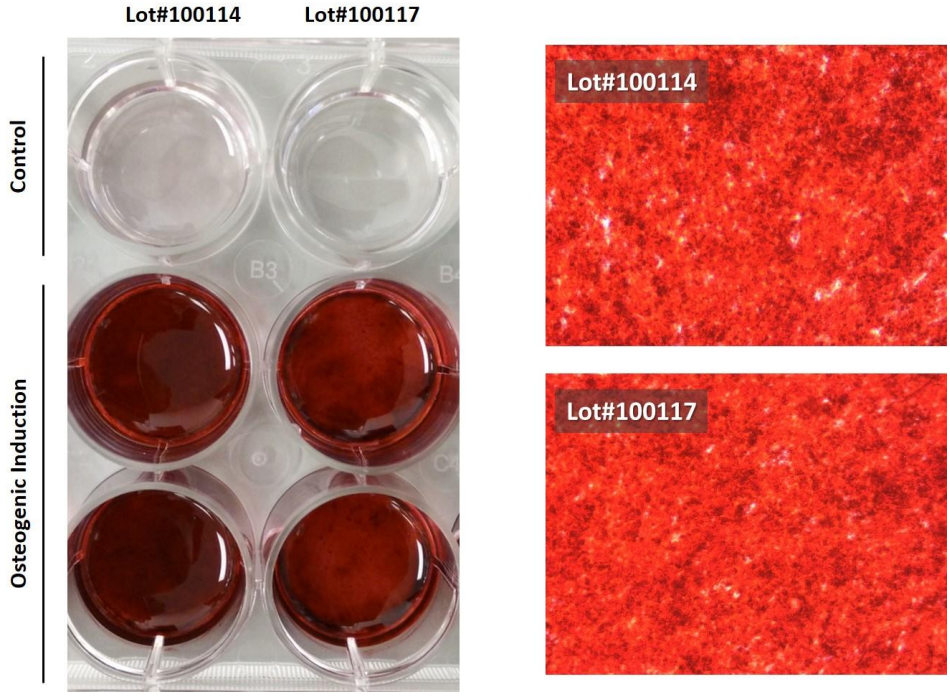

Figure 2. Mouse ADSCs (from white fat) can be differentiated to into osteoblasts using the osteogenic induction protocol (Alizarin Red S staining, Day 21).

6. Remove Alizarin Red S solution and immediately wash the cells 2~4 times with 1.5 ml ddH_2O. Carefully aspirate the wash buffer and apply 1 ml DPBS/well.
7. Analyze the cells. Undifferentiated ADSCs (without extracellular calcium deposits) are slightly reddish, while ADSC-derived osteoblasts (with extracellular deposits) are bright orange-red color (Figure 1 and 2).

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
Osteogenic Differentiation Medium	iXCells Biotechnologies	MD-0006
Alizarin Red S	Sigma	A5533

Disclaimers

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