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Product Information

Mouse Adipose-Derived Stem Cells

Catalog Number	10MU-005 (Interscapular Brown Fat) 10MU-006 (Inguinal White Fat)	Cell Number	0.5 million cells/vial
Species	*Mus musculus*	Storage Temperature	Liquid Nitrogen

Description

Adipose-derived stem cells (ADSC) are multipotent mesenchymal stem cells (MSC) that are capable of differentiating into adipocytes, osteocytes, chondrocytes etc *in vitro*. ADSC have been applied in studies including stem cell differentiation, regenerative medicine [1], cell therapy, tissue engineering and creation of iPS cell lines.

iXCells Biotechnologies provides high quality Mouse Adipose-Derived Stem Cells-brown fat (MADSC-bf), which are isolated from from C57BL/6 mouse inguinal white fat tissue or interscapular brown fat tissue. These cells are cryopreserved at P1, with >0.5 million cells in each vial and can further expand for 3-4 population doublings in Adipose-derived Stem Cell Growth Medium (Cat # MD-0003) under the condition suggested by iXCells Biotechnologies. In vitro, mADSC can be differentiated into adipocytes and osteoblasts (Figure 1 and 2) using Adipocyte Differentiation Medium (Cat # MD-0005) and Osteogenic Differentiation Medium (Cat # MD-0006). These mADSC are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast, and fungi.

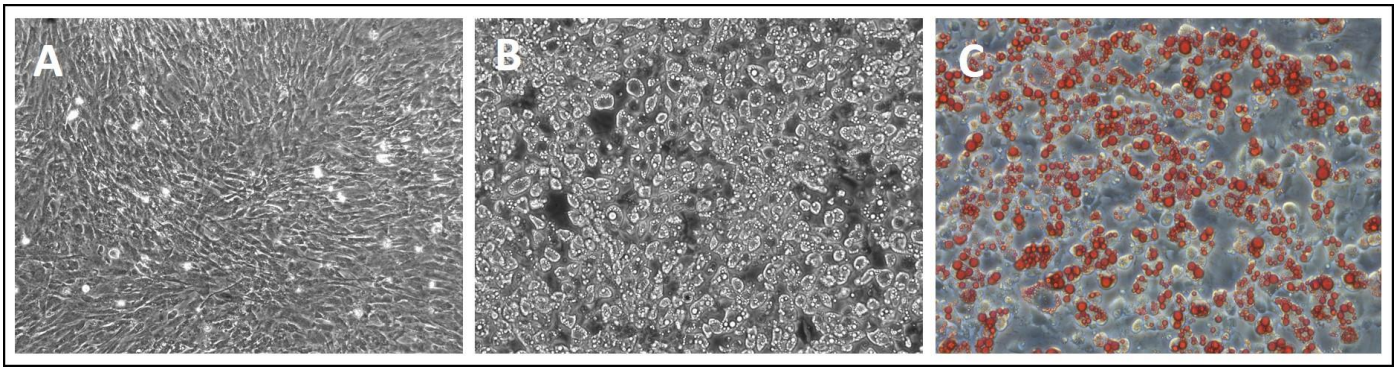

Figure 1. (A) Mouse ADSCs (phase contrast). **(B)** Adipocyte induction (Day 10 post adipogenic induction, phase contrast). **(C)** Adipocyte induction (Day 10 post adipogenic induction, Oil Red O staining).

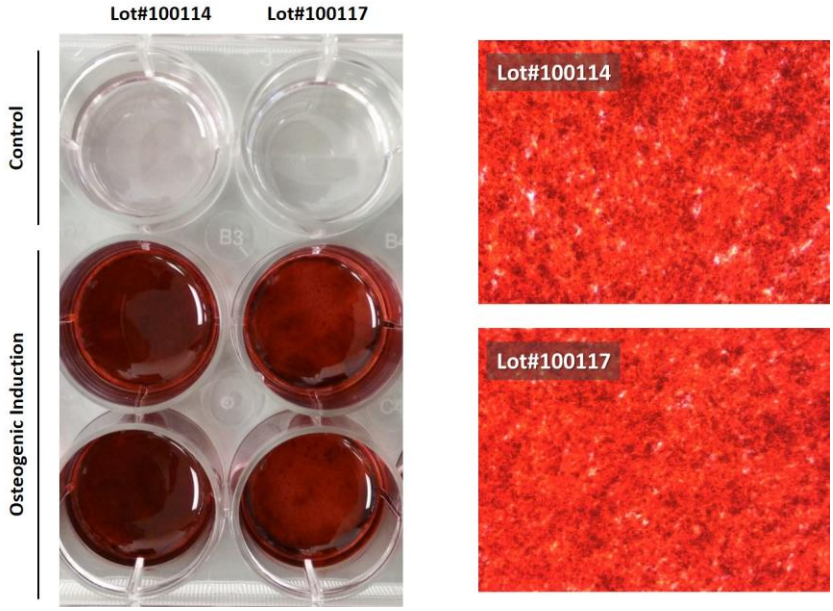

Figure 2. Mouse ADSCs can be differentiated to into osteoblasts using the osteogenic induction protocol (Alizarin Red S staining, Day 21 post osteogenic induction).

Product Details

Tissue	C57BL/6 mouse inguinal white fat or interscapular brown fat tissue
Package Size	0.5 x 10^6 cells/vial
Passage Number	P1
Shipped	Cryopreserved
Storage	Liquid nitrogen
Growth Properties	Adherent
Media	Adipose-derived Stem Cell Growth Medium (Cat # MD-0003) Adipocytes Differentiation Medium (Cat# MD-0005) Osteogenic Differentiation Medium (Cat# MD-0006)

Protocols

Thawing of Frozen Cells

1. Upon receipt of the frozen mADSCs, it is recommended to thaw the cells and initiate the culture immediately in order to retain the highest cell viability.
2. To thaw the cells, put the vial in 37°C water bath with gentle agitation for ~1 minute. Keep the cap out of water to minimize the

risk of contamination.

3. Pipette the cells into a 15ml conical tube with 5ml fresh Adipose-derived Stem Cell Growth Medium (Cat # MD-0003).
4. Centrifuge at 1,000rpm (~220g) for 5 minutes at room temperature.
5. Remove the supernatant and resuspend the cells in fresh Adipose-derived Stem Cells Growth Medium (Cat # MD-0003).
6. Culture the cell in T75 flask or 100mm dish.

Safety Precaution: *it is highly recommended that protective gloves and clothing should be used when handling frozen vials.*

Standard Culture Procedure

1. mADSCs can be cultured in Adipose-derived Stem Cell Growth Medium (Cat # MD-0003).
2. When cells reach ~80-90% confluence, remove the medium, and wash once with sterile PBS (5ml/T75 flask).
3. Add ~2.5ml of 0.25% Trypsin-EDTA to the flask and incubate for ~3 minutes at 37°C. Neutralize the enzyme by adding 2-3 volumes of cell Adipose-derived Stem Cell Growth Medium.
4. Centrifuge 1000rpm (~220g) for 5 minutes and resuspend the cells in desired volume of medium.
5. Seed new culture vessels at 5×10^3 cells/cm^2.

Adipocyte Differentiation Protocol (12 well plate format)

1. Grow mADSCs 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 mADSCs in Adipocytes Differentiation Medium for 7-10 days, and analyze the percentage of cells with oil-droplet formation by Oil Red O Staining (Figure 1).

Osteogenic Differentiation Protocol (12 well plate format)

1. Grow mADSCs in Adipose-Derived Stem Cell Growth Medium (Cat# MD-0003) to ~80% confluency.
2. Carefully aspirate the growth medium, then apply 1.5 ml Osteogenic Differentiation Medium per well (Cat# MD-0006) to the cells.
3. Culture the mADSCs for 2-3 weeks. Change fresh Osteogenic Differentiation Medium every 3 days. Be careful not to disturb the cell monolayer. The extracellular calcium deposit can be detected by Alizarin Red S staining (Figure 2).

References

[1] Harasymiak-Krzyzanowska I et al. Adipose tissue-derived stem cells show considerable promise for regenerative medicine applications. Cell Mol Biol Lett. 2013; 18(4): 479-493.

Disclaimers

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