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Human Placental Mesenchymal Stem Cells

Catalog Number	**10HU-170 (Decidua)** 10HU-171 (Chorionic villi) 10HU-172 (Chorionic plate)	**Cell Number**	0.5 million cells/vial
Species	*Homo sapiens*	**Storage Temperature**	Liquid Nitrogen

Description

Mesenchymal stem cells (MSC) derived from human placenta are a well-characterized population of adult stem cells. Number of researchers have shown that adult MSCs have a broad therapeutic potential due to their capability to renew and differentiate into various lineages of mature cells that produce fat, cartilage, bone, tendon, and muscle when cultured under specific permissive conditions [1]. In addition, placenta derived MSC show differentiation capacity toward both osteo-blasts and adipocytes thus serving as a great model for studying the molecular basis of differentiation [2]. These properties, in combination with their developmental plasticity, have generated tremendous interest in regenerative medicine to replace damaged tissues. These findings have spurred the development of MSC-based therapies for treating wide range of non-skeletal diseases [3].

iXCells Biotechnologies provides placental MSC isolated from different layers of human placenta, including decidua (Cat# 10HU-170), chorionic villi (Cat# 10HU-171), and chorionic plate (Cat# 10HU-172). Each vial contains ≥ 0.5 million cells. These cells are expanded in Mesenchymal Stem Cell Medium (Cat# MD-0037) and then cryopreserved at passage 2. The human placental MSC express typical mesenchymal cell surface markers, such as CD105, CD73, and CD90 and are negative for hematopoietic markers including CD34, CD45 and endothelial cell marker CD31 [Figure 1, 2]. These cells can further be differentiated into adipocytes using Adipocyte Differentiation Medium (Cat# MD-0005) and into osteoblasts using Osteogenic Differentiation Medium (Cat# MD-0006) [Figure 3 and Figure 4]. Human

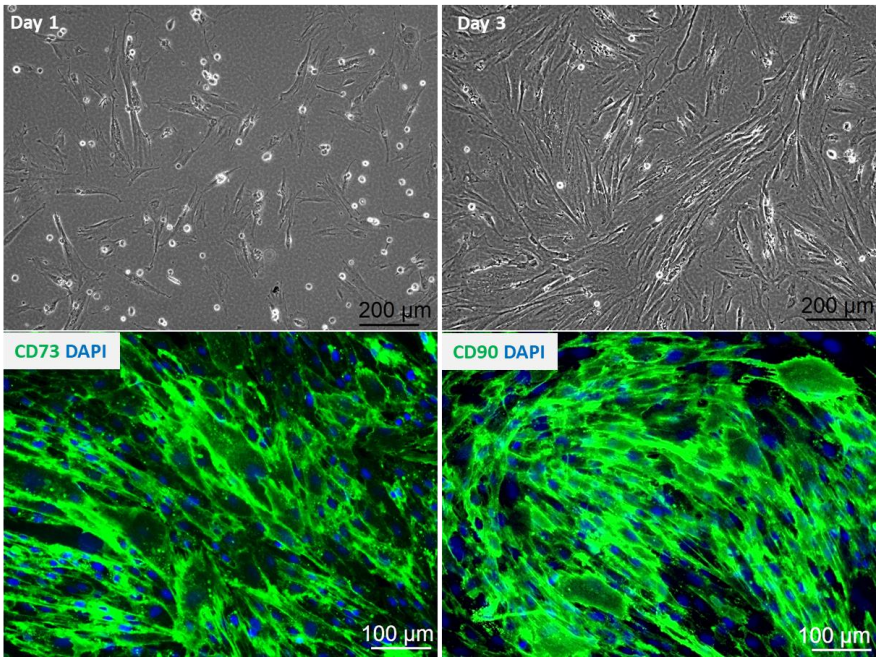

Figure 1. *Top*: Phase contrast images of human placental mesenchymal stem cell-Decidua (Cat# 10HU-170) taken at day 1 and day 3 post-recovery. *Bottom*: ICC staining using antibodies against CD73 (Green) and CD90 (Green), separately.

placental MSC are negative for mycoplasma, bacteria, yeast, and fungi and can be expanded for no more than 3 passages in iXCells' Mesenchymal Stem Cell Medium.

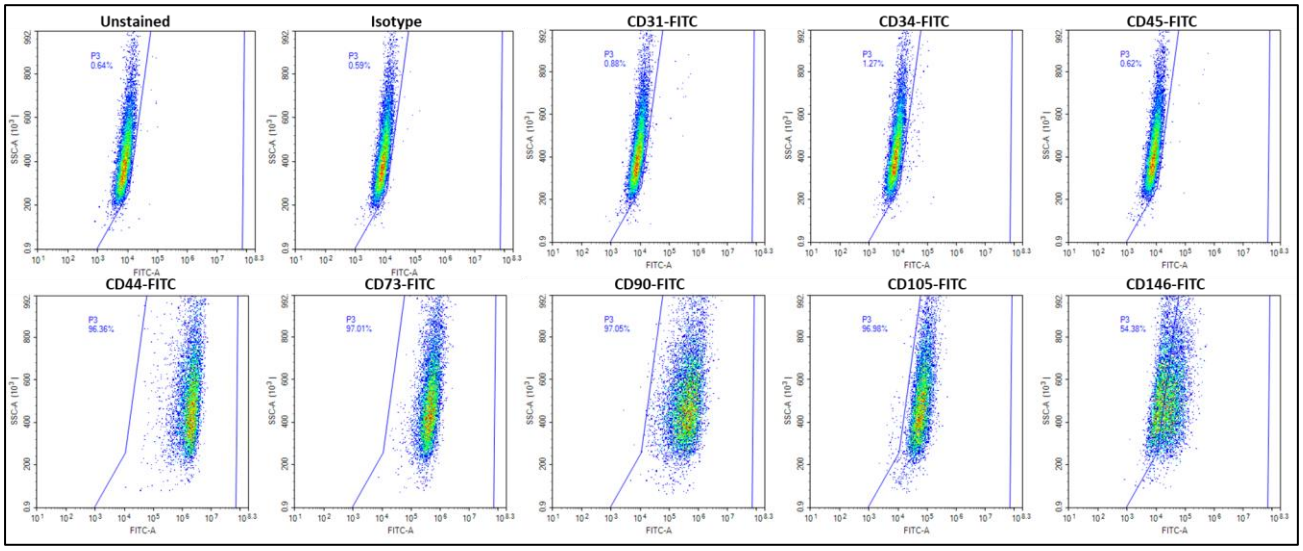

Figure 2. The purity of human placental mesenchymal stem cells-Decidua (Cat# 10HU-170) was determined using the corresponding antibodies by flow cytometry analysis. The sample stained with isotype control was used for gating strategy.

Product Details

Tissue	Human Placenta
Package Size	0.5 million cells/vial
Passage Number	P2
Shipped	Cryopreserved
Storage	Liquid nitrogen
Growth Properties	Adherent
Media	Mesenchymal Stem Cell Medium (Cat# MD-0037) Adipocyte Differentiation Medium (Cat# MD-0005) Osteogenic Differentiation Medium (Cat# MD-0006)

Protocol

Thawing of Frozen Cells

1. Upon receipt of the frozen human placental MSCs, 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 ~1-2 minutes. Keep the cap out of water to minimize the risk of contamination.
3. Pipette the cells into a 15 mL conical tube with 5 mL fresh **Mesenchymal Stem Cell Medium** (Cat# MD-0037).
4. Centrifuge at 1,000 rpm (~220 *g*) for 5 minutes under room temperature.

5. Remove the supernatant and re-suspend the cells in desired volume of fresh Mesenchymal Stem Cell Medium.
6. Culture the cells in one T75 flask. Change medium every 3~4 days until the cells reach about 80-90% confluence.

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

Standard Culture Procedure

1. When cells reach ~80-90% confluence, aspirate the culture medium and wash the cells with sterile PBS (5 mL/T75 flask).
2. Add ~2 mL of prewarm 0.25% Trypsin-EDTA to the flask and incubate for ~3 minutes at 37°C. When majority of cells have detached from the surface, neutralize the enzyme by adding 2-3 volumes of **Mesenchymal Stem Cell Medium** (Cat# MD-0037).
3. Gently pipette and collect the dissociated cells into a sterile centrifuge tube.
4. Add another 3 mL of cell culture medium to dislodge the remaining cells and transfer detached cells into the same tube.
5. Centrifuge the tube at 1,000 rpm (~220 *g*) for 5 minutes and re-suspend the cells in desired volume of medium.
6. Count and seed the cells at the recommended cell density (5,000-7000 viable cells/cm^2). Change the medium every 3-4 days until the cells are confluent.

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

Adipocytes Differentiation Protocol

1. Culture human placental MSCs in a 6-well plate using **Mesenchymal Stem Cell Medium** (Cat# MD-0037) until cells reach >95% confluence.
2. Aspirate the growth medium and replace with 1.5 mL fresh growth medium/well. Let the cells grow for 2-3 additional days.

   Note*: Cells at this stage may detach from dish easily. Use pipet and slowly remove the medium. Add Adipocyte Differentiation Medium slowly to avoid cell detachment.*
3. Remove the Mesenchymal Stem Cell Medium and apply 1.5 mL **Adipocyte Differentiation Medium** (Cat# MD-0005) per well.
4. Change adipocyte differentiation medium every 3~4 days for up to 4 weeks. The accumulation of lipid droplets in cytoplasm will appear after 3 week which can be analyzed by Oil Red O staining (Figure 3).

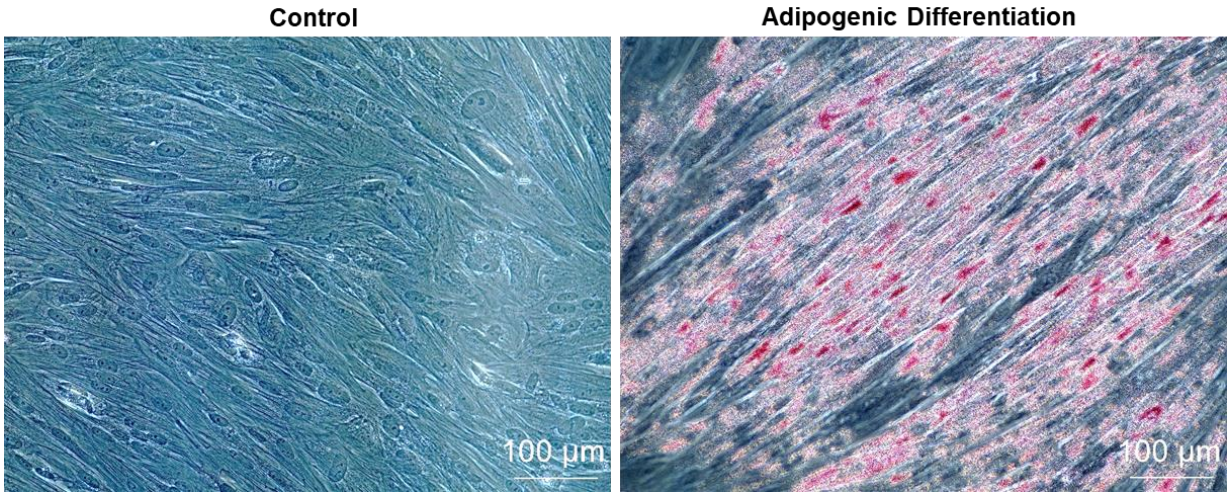

Figure 3. Human placental mesenchymal stem cells-Decidua (Cat# 10HU-170) adipocyte differentiation (Day 22 post adipocyte induction).

Osteogenic Differentiation Protocol

1. Culture human placental MSCs in a 6-well plate in **Mesenchymal Stem Cell Medium** (Cat# MD-0037) until cells reach ~80% confluence.
2. Carefully aspirate growth medium, apply 1.5 mL **Osteogenic Differentiation Medium** (Cat# MD-0006) per well.
3. Change fresh Osteogenic Differentiation Medium every 3 days.

   ***Note**: Cells at this stage may detach from dish easily. Use pipet and slowly remove the medium. Add Osteogenic Differentiation Medium slowly to avoid cell detachment.*
4. Culture the cells for 2-3 weeks and osteoblasts can be detected by Alizarin Red S staining (Figure 4).

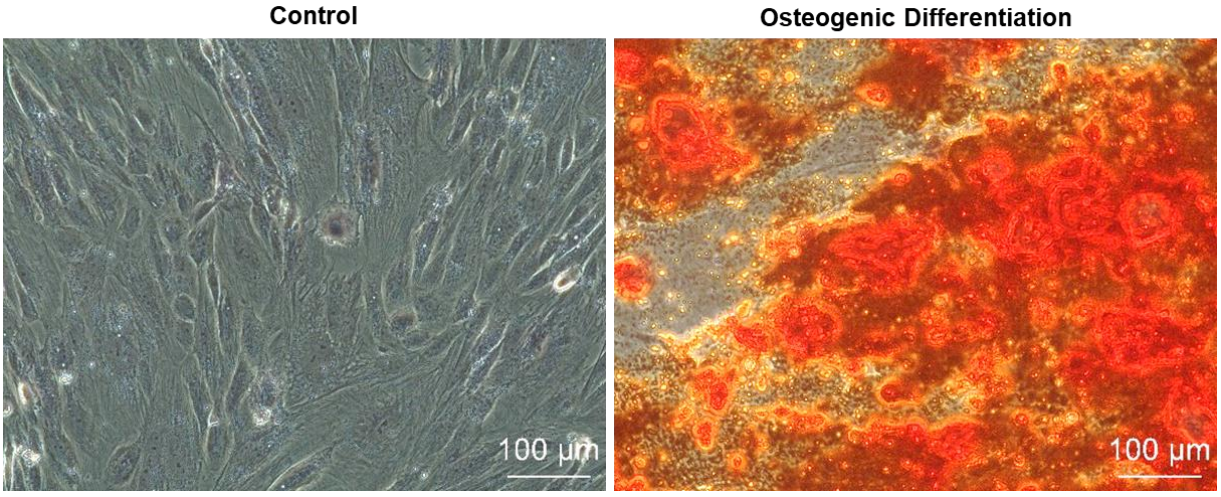

Figure 4. Osteogenic differentiation (Day 20 post osteocyte induction) using human placental mesenchymal stem cells -Decidua (Cat# 10HU-170). Alizarin Red S staining of osteoblasts. The extracellular calcium deposit was stained in bright orange-red color.

References

[1] Miao, Z., Jin, J., Chen, L., Zhu, J., Huang, W., Zhao, J., Qian, H., & Zhang, X. (2006). Isolation of mesenchymal stem cells from human placenta: comparison with human bone marrow mesenchymal stem cells. Cell biology international, 30(9), 681–687.

[2] Fukuchi, Y., Nakajima, H., Sugiyama, D., Hirose, I., Kitamura, T., & Tsuji, K. (2004). Human placenta-derived cells have mesenchymal stem/progenitor cell potential. Stem cells (Dayton, Ohio), 22(5), 649–658.

[3] García-Gómez I, Elvira G, Zapata AG, Lamana ML, Ramírez M, Castro JG, Arranz MG, Vicente A, Bueren J, García-Olmo D.(2010) Mesenchymal stem cells: biological properties and clinical applications. Expert Opin Biol Ther. 10(10):1453-68.

Disclaimers