Human Umbilical Mesenchymal Stem Cells (HUMSC)

Catalog #7530

Cell Specification

Mesenchymal stem cells (MSC) are a well-characterized population of adult stem cells. MSC have the potential to develop into mature cells that produce fat, cartilage, bone, tendons, and muscle. These properties, in combination with their developmental plasticity, have generated tremendous interest because of the potential use of MSC in regenerative medicine. MSC isolated from the Wharton's jelly of the umbilical cord were induced to transform into neurons and glia *in vitro* by using neuron-conditioned medium, sonic hedgehog, and FGF-8 [1, 2]. MSC can also differentiate into cells from the adipogenic and osteogenic lineage. Additionally, they have the potential to differentiate into cardiomyocytes by culturing them in cardiomyocyte-conditioned medium [3]. MSC express the matrix receptors CD44 and CD105 and mesenchymal stem cell markers SH2 and SH3, but not the hematopoietic lineage marker CD34.

HUMSC from ScienCell Research Laboratories are isolated from Wharton's jelly of the umbilical cord. HUMSC are cryopreserved at passage one and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. HUMSC are characterized by immunofluorescence with antibodies specific to CD73, CD90 and CD105. HUMSC are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi. HUMSC are guaranteed to further expand for 15 population doublings under the conditions provided by ScienCell Research Laboratories.

Recommended Medium

It is recommended to use Mesenchymal Stem Cell Medium (MSCM, Cat. #7501) for culturing HUMSC *in vitro*.

Product Use

HUMSC are for research use only. They are not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Upon receiving, directly and immediately transfer the cells from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments.

Shipping

Dry ice.

References

[1] Mitchell KE, Weiss ML, Mitchell BM, Martin P, Davis D, Morales L, Helwig B, Beerenstrauch M, Abou-Easa K, Hildreth T, Troyer D, Medicetty S. (2003) "Matrix cells from Wharton's jelly form neurons and glia." *Stem Cells* 21: 50-60.

[2] Fu YS, Cheng YC, Lin MY, Cheng H, Chu PM, Chou SC, Shih YH, Ko MH, Sung MS. (2006) "Conversion of human umbilical cord mesenchymal stem cells in Wharton's jelly to dopaminergic neurons in vitro: potential therapeutic application for Parkinsonism." *Stem Cells* 24: 115-124.

[3] Wang HS, Hung SC, Peng ST, Huang CC, Wei HM, Guo YJ, Fu YS, Lai MC, Chen CC. (2004) "Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord." *Stem cells* 22: 1330-1337.

Instructions for culturing cells

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37°C water bath and return the cells to culture as quickly as possible with minimal handling!

Initiating the culture:

1. Prepare a fibronectin-coated culture vessel (2 µg/cm^2, T-75 flask is recommended). Add 5 ml of sterile Dulbecco's phosphate buffered saline, Ca^{++}- and Mg^{++}-free (Cat. #0303) to a T-75 flask and then add 150 µl of fibronectin stock solution (Cat. #8248). Leave the vessel in a 37°C incubator overnight.
2. Prepare complete medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Aseptically transfer supplement to the basal medium with a pipette. Rinse the supplement tube with medium to recover the entire volume.
3. Aspirate fibronectin solution and add 15 ml of complete medium to the culture vessel. The fibronectin solution can be used twice. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.
4. Place the frozen vial in a 37°C water bath. Hold and rotate the vial gently until the contents completely thaw. Promptly remove the vial from the water bath, wipe it down with 70% ethanol, and transfer it to the sterile field.
5. Carefully remove the cap without touching the interior threads. Gently resuspend and dispense the contents of the vial into the equilibrated, fibronectin-coated culture vessel. A seeding density of 5,000 cells/cm^2 is recommended.

   Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of residual DMSO in the culture. It is also important that cells are plated in fibronectin-coated culture vessels to promote cell attachment.
6. Replace the cap or lid of the culture vessel and gently rock the vessel to distribute the cells evenly. Loosen cap, if necessary, to allow gas exchange.
7. Return the culture vessel to the incubator.
8. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove residual DMSO and unattached cells.
9. A healthy culture will display fibroblast-like morphology, usually in scattered single cells rather than a homogeneous bundle or sheet of cells. Cell number will double after two to three days in culture.

Maintaining the culture:

1. Refresh supplemented culture medium the next morning after establishing a culture from cryopreserved cells.
2. Change the medium every 48 hours thereafter, until the culture is approximately 50% confluent.

3. Once the culture reaches 50% confluency, change medium every day until the culture is approximately 90% confluent.

Subculturing:

1. Subculture when the culture reaches 90-95% confluency.
2. Prepare fibronectin-coated culture vessels (2 μg/cm^2) one day before subculture.
3. Warm complete medium, trypsin/EDTA solution (T/E, Cat. #0103), T/E neutralization solution (TNS, Cat. #0113), and DPBS (Ca^{++}- and Mg^{++} -free, Cat. #0303) to **room temperature**. We do not recommend warming reagents and medium in a 37^{o}C water bath prior to use.
4. Rinse the cells with DPBS.
5. Add 10 ml of DPBS and then 2 ml of T/E solution into flask (in the case of a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Incubate the flask in a 37^{o}C incubator for 1 to 2 minutes or until cells completely round up. Use a microscope to monitor the change in cell morphology.
6. During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, Cat. #0500).
7. Transfer T/E solution from the flask to the 50 ml centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37^{o}C for another 1 minute (no solution in the flask at this moment).
8. At the end of incubation, gently tap the side of the flask to dislodge cells from the surface. Check under a microscope to make sure that all cells detach.
9. Add 5 ml of TNS solution to the flask and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5 ml of TNS to collect the residual cells.
10. Examine the flask under a microscope for a successful cell harvest by looking at the number of cells being left behind; there should be less than 5%.

    Note: Use ScienCell T/E solution that is optimized to minimize cell damages due to over trypsinization.
11. Centrifuge the 50 ml centrifuge tube at 1000 rpm for 5 minutes. Resuspend cells in culture medium.
12. Count and plate cells in a new fibronectin-coated culture vessel with the recommended cell density.

Caution: Handling human derived products is potentially biohazardous. Although each cell strain tests negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working with these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

[1] Grizzle WE, Polt S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Cult Methods*. 11: 191-9.