

Human Mesenchymal Stem Cells - Adipose Tissue | 300645

General information

Description MSCs, or multipotent mesenchymal stromal cells, are self-renewing multipotent cells that can differentiate into a wide variety of cell types. The in vitro direct differentiation of MSCs into at least three orthodoxal lineages- adipocytes, osteoblasts, and chondrocytes has been demonstrated. Using differentiation media, it is possible to differentiate cultivated MSCs into adipocytes, osteoblasts, and chondrocytes in vitro. Early passage cultured MSCs are cryopreserved using a specific cryomedium. After thawing, each cryovial contains 1×10^6 (5% minimum 92% to 95% viability level by Trypan Blue dye exclusion test). The MSCs were collected from healthy donors which have given their informed consent for the donation of the cell material. Each batch of MSCs is subjected to strict quality control testing (both cell donors and cell cultures). The identification, purity, potency, viability, and appropriateness of cultured MSCs for the intended usage are all evaluated.

Organism Human

Tissue Adipose Tissue

Applications Drug testing, regenerative medicine, disease research

Characteristics

Age Please inquire

Gender Please inquire

Ethnicity Caucasian

Morphology Well-spread spindle shaped, fibroblast-like morphology for at least within 5 passages. Fewer than 2% cells exhibit spontaneous myofibroblast-like morphology within each passage.

Cell type Stem cell

Growth properties Adherent

Identifiers / Biosafety / Citation

Citation Human Mesenchymal Stem Cells, Adipose Tissue (Cytion catalog number 300645)

Biosafety level 1

Expression / Mutation

Human Mesenchymal Stem Cells - Adipose Tissue | 300645

Antigen expression A comprehensive panel of markers, including CD73/CD90/CD105 (positive) and CD14/CD34/CD45/HLA-DR (negative), are used in flow cytometry analysis to identify cultivated MSCs (P2-P3) prior to cryopreservation. These markers are recommended by the ISCT MSC committee.

Viruses Donor is negative for HBV (PCR), Treponema pallidum (PCR), and HIV-1/2 (IFA). Cells are negative for HBV, HCV, HSV1, HSV2, CMV, EBV, HHV6, Toxoplasma gondii, Treponema pallidum, Chlamydia trachomatis, Ureaplasma urealyticum, and Ureaplasma parvum.

Handling

Culture Medium Alpha MEM

Medium supplements 10% FBS, 2 ng/ml bFGF, w: 2.0 mM stable Glutamine, w/o: Ribonucleosides, w/o: Deoxyribonucleosides, w: 1.0 mM Sodium pyruvate, w: 2.2g/L NaHCO₃

Passaging solution Trypsin-EDTA

Subculturing Remove medium and rinse the adherent cells using PBS without calcium and magnesium (3-5 ml PBS for T25, 5-10 ml for T75 cell culture flasks). Add Accutase (1-2 ml per T25, 2.5 ml per T75 cell culture flask), the cell sheet must be covered completely. Incubate at ambient temperature for 8-10 minutes. Carefully resuspend the cells with medium (10 ml), centrifuge for 3 min at 300 g, resuspend cells in fresh medium and dispense into new flasks which contain fresh medium.

Seeding density 1 to 3 x 10⁴ cells/cm²

Fluid renewal First fluid renewal after 24 hours, then every 2 to 3 days.

Freeze medium CM-1 (Cytion catalog number 800100) or CM-ACF (Cytion catalog number 806100)

Human Mesenchymal Stem Cells - Adipose Tissue | 300645

Handling of cryopreserved cultures

The cells come deep-frozen shipped on dry ice. Please make sure that the vial is still frozen. If immediate culturing is not intended, the cryovial must be stored below -150 degree Celsius after arrival. If immediate culturing is intended, please follow the below instructions: Quickly thaw by rapid agitation in a 37 degree Celsius water bath within 40-60 seconds. The water bath should have clean water containing an antimicrobial agent. As soon as the sample has thawed, remove the cryovial from the water bath. A small ice clump should still remain and the vial should still be cold. From now on, all operations should be carried out under aseptic conditions. Transfer the cryovial to a sterile flow cabinet and wipe with 70% alcohol. Carefully open the vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of culture medium (room temperature). Resuspend the cells carefully. Centrifuge at 300 x g for 3 min and discard the supernatant. The centrifugation step may be omitted, but in this case the remains of the freeze medium have to be removed 24 hours later. Calculate the needed culture surface area according to the plating density (for MSCs, as a rule 2 to 3×10³ / cm²). Resuspend the cells carefully in an appropriate amount of fresh cell culture medium and transfer them into two cell culture flasks. All further steps are described in the subculture section.

Handling of proliferating cultures

One or two cell culture flasks come filled with cell culture medium. Collect the entire medium in 1 or 2 x 50 ml centrifuge tubes, respectively. Carefully add 5 ml of cell culture medium to each T25 cell culture flask. Control the cell morphology and confluency under the microscope. Incubate at 37 degree Celsius for a minimum of 24 hours. Spin down the collected medium at 300 x g for 3 minutes to collect the cells which may have detached during transit. If a cell pellet is visible, resuspend the cells in 5 ml of cell culture medium and transfer to a T25 cell culture flask. Incubate at 37 degree Celsius for a minimum of 24 hours.

Quality control / Genetic profile / HLA

Sterility

Mycoplasma contaminations are ruled out through PCR-based and luminescence-based mycoplasma assays. The absence of bacterial, fungal or yeast contamination is controlled through daily visual cell monitoring.