Life Technologies™
Your Molecular & Cell Technology Partner

TOTAL KIT: HUMAN MESENCHYMAL STEM CELLS DERIVED FROM ADIPOSE TISSUE

Catalog Number: hMSC-AT-TK-om

Product description:

Mesenchymal stem cells belong to a well characterized population of adult stem cells. hMSC are isolated from adipose tissue are fibroblast-like cells. They are multipotent in nature as they can differentiate into cells from osteogenic, chondrogenic, odontogenic, adipogenic, myogenic and neurogenic lineage. Hence, able to proliferate producing bone, tendons, muscle, cartilage and fat. These cells find their applications in tissue forming using 3D printed scaffolds. Their proliferation efficiency has opened new avenues in cell-based therapy in restoration of diseases or damaged tissue and cells are utilized in regenerative medicines. They are used in many research applications involving neurodegenerative, cardiovascular, autoimmune, bone and cartilage diseases because of their properties of anti-fibrotic and anti-inflammatory capabilities.

Characterization: Immunofluorescence with antibodies specific to CD73 and/or CD90, Oil Red O staining after adipogenic differentiation, and Alizarin Red staining after osteogenic differentiation.

Composition:

Cryovial hMSC (cat #: hMSC-AT)	$1x10^6$ Cells/vial
Growth Medium (cat #: hMSC-AT-GM-100)	100 mL
Dissociation solution (cat #: hMSC-DS-10)	10 mL

Specifications:

Tissue	Normal healthy human adipose tissue
Cell type	Mesenchymal stem cells
Description	Human mesenchymal stem cells derived from adipose tissue
Alternative names	hMSC
Application	Stem cell research, producing bone, tendons, muscle, cartilage and fat tissue formation using 3D printed scaffolds.
Product use	This product is for research use only.
Storage	Cryopreserved vials: store in liquid nitrogen immediately after receiving. Growth medium: store in 4ºC in dark immediately after receiving. Dissociation solution: store in -20ºC
Shelf life	Growth medium: 1 month at 4ºC. Dissociation solution: 3 months at -20 ºC
Shipping	Cells: Dry ice Growth medium: 4ºC. Dissociation solution: Dry ice
QC	Tested negative for bacteria, fungi, yeast. Tested negative for mycoplasma, endotoxin. Tested negative for Hepatitis A, B, C and HIV 1 and 2 viruses.

Instructions to use:

Growth medium should be thawed at 37 ºC and mixed thoroughly before use.
Dissociation solution should be thawed at 37 ºC before use.
The recommended concentration of penicillin/streptomycin Antibiotic Solution is 50 I.U./mL penicillin and 50µg/mL streptomycin.

Note: Growth medium and dissociation solution once opened should be used before a month.

Life Technologies® is a registered trademark of Life Technologies (India) Pvt Ltd.

PROTOCOL FOR SEEDING, MAINTENANCE, SUBCULTURING AND CRYOPRESERVATION OF HUMAN ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELLS

I. SEEDING OF HUMAN ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELLS FROM FROZEN AMPULE

MATERIALS

i. Human adipose tissue derived mesenchymal stem cell basal medium (1X) (Catalog #hMSC-AT-GM-500)
ii. Human adipose tissue derived mesenchymal stem cell growth supplement (Catalog #hMSC-AT-GS-50)
iii. Human adipose tissue derived mesenchymal stem cells (Catalog #hMSC-AT)
iv. T25 flask
v. 10 to 1000µl pipette tips
vi. 37°C incubator with a humidified atmosphere of 5% CO_2
vii. 5ml centrifuge tubes
viii. Adjustable pipettes (2-10µl, 10-100µl, and 100-1000µl), multichannel pipettes and a pipettor.
ix. Antibiotic-antimycotic solution

METHODS

Note:

- Typically, human adipose tissue derived mesenchymal stem cells take 24-36hrs to proliferate.
- Human adipose tissue derived mesenchymal stem cells can undergo 6-8 passaging.
- Surface sterilize the biosafety cabinet and required materials with 70% ethanol.
- Prewarm the human adipose tissue derived mesenchymal stem cell basal medium and human adipose tissue derived mesenchymal stem cells growth supplement in water bath at 37°C for 15 minutes before starting the protocol.
- Prepare human adipose tissue derived mesenchymal stem cell complete growth medium by mixing 10% human adipose tissue derived mesenchymal stem cell growth supplement with human adipose tissue derived mesenchymal stem cells basal medium (1X).

Life Technologies® is a registered trademark of Life Technologies (India) Pvt Ltd.

a. Take the cryovial from the cryopreservation tank and thaw the cells in 37°C water bath, by holding the vial partially submerged and swirling the vial constantly. **Ideally, thawing time should be one or two minutes.**

b. Once thawed, immediately remove the vial from the water bath and wipe the vial with 70% ethanol. Perform below prescribed procedure using aseptic techniques inside biosafety cabinet (Class II type A2).

c. **After thawing the cells make sure to process them immediately.** Leaving the cells at room temperature for an extended period, without processing them, will reduce the viability of the cells.

d. Transfer the cells directly into a 5ml sterile centrifuge tube and then bring the total volume to 2 ml using human adipose tissue derived mesenchymal stem cells growth medium.

e. Centrifuge the cells to a soft pellet at 1800rpm for 5 minutes.

f. Remove the supernatant and resuspend the cells in a total volume of 1ml complete growth medium and add to T25 flask, having 4ml of complete growth media. Addition of antibiotic-antimycotic solution into the flask is recommended.

g. Place the flask into a humidified tissue culture incubator at 37°C and 5% CO_2.

h. Cells should start to attach after 12 hours and should be completely attached in 24-36 hrs.

II. MAINTENANCE OF HUMAN ADIPOSE TISSSUE DERIVED MESENCHYMAL STEM CELLS

MATERIALS:

i. Human adipose tissue derived mesenchymal stem cells (Catalog #hMSC-AT).

ii. Human adipose tissue derived mesenchymal stem cell basal medium (1X) (Catalog #hMSC-AT-GM-500)

iii. Human adipose tissue derived mesenchymal stem cell growth supplement (Catalog #hMSC-AT-GS-50)

iv. 10 to 1000 µl pipette tips

v. 37°C incubator with a humidified atmosphere of 5% CO_2

vi. 5ml centrifuge tubes

vii. Adjustable pipettes (2-10µl, 10-100µl, and 100-1000µl), multichannel pipettes and a pipettor.

viii. Antibiotic-antimycotic solution

Life Technologies® is a registered trademark of Life Technologies (India) Pvt Ltd.

ix. D-PBS (Ca+ and Mg+ free)

METHODS:

Note:

- Prepare human adipose tissue derived mesenchymal stem cell complete growth medium by mixing 10% human adipose tissue derived mesenchymal stem cell growth supplement with human adipose tissue derived mesenchymal stem cells basal medium (1X).
- Perform the below prescribed procedure in sterilized condition inside biosafety cabinet (Class II type A2).

a. When visual inspection shows a majority of the cells attached (approximately 80%), remove the spent human adipose tissue derived mesenchymal stem cell complete growth medium and rinse the cell monolayer with 1-2 ml of sterile D-PBS to remove all traces of serum. Replenish the flask with fresh complete growth medium.
b. Feed cells with fresh complete growth medium at every alternative day and treat with recommended antibiotic-antimycotic solution as per requirement.
c. When the cells have reached 75 -80% confluency, subculture the cells (the suggested splitting ratio is 1:2).

III. SUBCULTURING OF HUMAN ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELLS

MATERIALS:

i. Human adipose tissue derived mesenchymal stem cells (Catalog #hMSC-AT)
ii. Human adipose tissue derived mesenchymal stem cell basal medium (1X) (Catalog #hMSC-AT-GM-500)
iii. Human adipose tissue derived mesenchymal stem cell growth supplement (Catalog #hMSC-AT-GS-50)
iv. T25 flask
v. 10 to 1000 µl pipette tips
vi. 37°C incubator with a humidified atmosphere of 5% CO_2
vii. 5ml centrifuge tubes
viii. Adjustable pipettes (2-10µl, 10-100µl, and 100-1000µl), multichannel pipettes and a

Life Technologies® is a registered trademark of Life Technologies (India) Pvt Ltd.

pipettor.

ix. Antibiotic-antimycotic solution
x. D-PBS (Ca+ and Mg+ free)
xi. Trypsin-EDTA solution

METHODS:

Note:

- Prepare human adipose tissue derived mesenchymal stem cells complete growth medium by mixing 10% human adipose tissue derived mesenchymal stem cell growth supplement with human adipose tissue derived mesenchymal stem cell basal medium (1X).

a. Perform the below prescribed procedure in sterilized condition inside a biosafety cabinet (Class II type A2).
b. When the cells reach 75 -80% confluency, subculture the cells for expansion.
c. Using a sterile pipette, discard the spent culture media. Any materials and solutions coming intocontact with cells should always be disposed of properly.
d. Rinse the cell monolayer with 1-2 ml of sterile D-PBS to remove all traces of serum.
e. Add 1 ml of Trypsin-EDTA solution to the T25 flask and incubate at 37°C for 2 to 3 mins. Checkthe progress of the enzyme treatment every few minutes on an inverted phase-contrast microscope.
f. Once the cells are detached, add 2 ml of fresh human adipose tissue derived mesenchymal stem cells growth complete medium to the T25 flask to inactivate the Trypsin-EDTA solution. Make a homogenate suspension of single cells by vigorous pipetting, ensuring washing off any remaining attached-cells from thebottom of the culture flask.
g. Collect the suspended cells in a sterile 5 ml centrifuge tube and then centrifuge at 1800rpm for 5 minutes to obtain a soft cell pellet.
h. Resuspend the cell pellet in 1ml of fresh growth media making single cell suspension with gentlepipetting.
i. Count the cells by using a hemocytometer and trypan blue solution to check cell viability.
j. Plate the cells at $1x10^5$ cells per ml density into fresh flask. Add recommended antibiotic-antimycotic solution into the flask as per requirement.

Life Technologies® is a registered trademark of Life Technologies (India) Pvt Ltd.

IV. CRYOPRESERVATION OF CELLS:

MATERIALS:

i. Human adipose tissue derived Mesenchymal Stem Cells (Catalog #hMSC-AT)
ii. Human adipose tissue derived mesenchymal stem cell basal medium (1X) (Catalog #hMSC-AT-GM-500).
iii. Human adipose tissue derived mesenchymal stem cell growth supplement (Catalog #hMSC-AT-GS-50).
iv. 10 to 1000 µl pipette tips
v. 37°C incubator with a humidified atmosphere of 5% CO_2
vi. 5ml centrifuge tubes
vii. Adjustable pipettes (2-10µl, 10-100µl, and 100-1000µl), multichannel pipettes and a pipettor
viii. Antibiotic-antimycotic solution
ix. Cryovial freezing container (pre-chilled)
x. DMSO
xi. D-PBS (Ca+ and Mg+ free)
xii. Fetal Bovine Serum
xiii. Sterile cryogenic vial (standing)
xiv. Trypsin-EDTA solution

METHODS:

Note:

- Cells that are healthy and rapidly dividing to be frozen.
- Prepare human adipose tissue derived mesenchymal stem cells complete growth medium by mixing 10% human adipose tissue derived mesenchymal stem cell growth supplement with human adipose tissue derived mesenchymal stem cell basal medium (1X).
- **Freezing media composition: 10% DMSO + 40% FBS + 50% Complete growth media.**

a. Culture selection and examination: Prior to freezing, the culture should be maintained in an actively growing state (log phase or exponential growth) to ensure optimum health

Life Technologies® is a registered trademark of Life Technologies (India) Pvt Ltd.

and good recovery. Ideally, the culture medium should be changed 24 hours prior to harvesting.

b. Using sterile pipette, remove and discard the spent culture medium. Any materials and solutions coming into contact with cells should always be disposed of properly.
c. Rinse the cell monolayer with 1-2 ml of sterile 1X PBS solution to remove all traces of serum.
d. Add 1 ml of Trypsin-EDTA solution to the T25 flask, and incubate at 37°C in a 5% CO_2 incubator for 2 to 3 minutes. Check the progress of the enzyme treatment every few minutes on an inverted phase-contrast microscope.
e. Once the cells are detached, add 2 ml of fresh human adipose tissue derived mesenchymal stem cells complete growth mediumto the T25 flask to inactivate the Trypsin-EDTA solution. Make a homogenate suspension of single cells by vigorous pipetting ensuring washing off any remaining cells from the bottom of the culture flask.
f. Collect the suspended cells in a 5 ml centrifuge tube and then centrifuge at 1800rpm for 5 minutes to obtain a soft cell pellet.
g. Remove the supernatant and resuspend the cell pellet in 1ml culture media. Count the cells by using a hemocytometer and trypan blue solution to check cell viability. Centrifuge the cell suspension and remove the supernatant.
h. Add the required amount of cell freezing media (10% DMSO + 40% FBS + 50% complete growth media)to get the cell count to 1 million cells per ml.
i. Label the appropriate number of plastic cryogenic vials with at least the name of the cell and the date.
j. Add 1 ml of the cell suspension to each of the vials and seal.
k. Freezing cells: Place the cryovials in a freezing container (pre-chilled) at -80°C for overnight. A slow and reproducible cooling rate is very important to ensure good recovery of cultures. A decrease of -1 to -3°C / minute is recommended.
l. Next day, transfer the cryovials into a vapor phase of liquid nitrogen condition.

Life Technologies® is a registered trademark of Life Technologies (India) Pvt Ltd.