Mesenchymal Stem Cells

PromoCell

Instruction Manual

Product	Size	Catalog Number
Human Mesenchymal Stem Cells from Bone Marrow (hMSC-BM)	500,000 cryopreserved cells 500,000 proliferating cells	C-12974 C-12975
Human Mesenchymal Stem Cells from Umbilical Cord Matrix (hMSC-UC)	500,000 cryopreserved cells 500,000 proliferating cells	C-12971 C-12972
Human Mesenchymal Stem Cells from Adipose Tissue (hMSC-AT)	500,000 cryopreserved cells 500,000 proliferating cells	C-12977 C-12978

Product Description

Mesenchymal Stem Cells (MSC), also termed Mesenchymal Stromal Cells, are self-renewing multipotent cells that can differentiate into a wide variety of cell types. MSC have been shown to differentiate *in vitro* into adipocytes, chondrocytes, osteoblasts, myocytes, and ß-pancreatic islets cells. They can also transdifferentiate into neuronal cells and hepatocytes.

PromoCell offers a range of Mesenchymal Stem Cells produced at PromoCell's cell culture facility from normal human tissues of different origins. Differentiation of MSC into adipocytes, osteoblasts, chondrocytes, and neuronal lineages can be performed using PromoCell Mesenchymal Stem Cell Differentiation Media system (see Instruction Manual "Mesenchymal Stem Cell Media").

Shortly after isolation, all Mesenchymal Stem Cells are cryopreserved using PromoCell's proprietary, serum-free freezing medium, Cryo-SFM. Thawing and seeding results in passage 2.

Each cryo vial contains more than 500,000 viable cells after thawing.

Proliferating cell cultures are made from 500,000 cryopreserved cells that have been thawed and cultured for three days at PromoCell.

Quality Control

Rigid quality control tests are performed for each lot of PromoCell Mesenchymal Stem Cells.

They are tested for cell morphology, adherence rate, and viability. Furthermore, they are characterized by flow cytometric analysis of a comprehensive panel of markers, e.g. PECAM (CD31), HCAM (CD44), CD45, and Endoglin (CD105).

Differentiation assays into adipogenic, osteogenic, chrondrogenic and neurogenic lineages are performed for each lot under culture conditions without antibiotics and antimycotics. Differentiation performance is guaranteed up to 10 population doublings (PD).

In addition, all cells have been tested for the absence of HIV-1, HIV-2, HBV, HCV, and microbial contaminants (fungi, bacteria, and mycoplasma).

A detailed certificate of analysis (CoA) for each lot can be downloaded at: www.promocell.com/coa

Intended Use

PromoCell Mesenchymal Stem Cells are for *in vitro* research use only and not for diagnostic or therapeutic procedures.

Warning

Although tested negative for HIV-1, HIV-2, HBV, and HCV, the cells - like all products of human origin - should be handled as potentially infectious. No test procedure can completely guarantee the absence of infectious agents.

Follow appropriate safety precautions!

After delivery, start immediately with the protocol for cryopreserved cells (see page 2) or the protocol for proliferating cells (see page 3).

Start immediately after delivery.
Use aseptic techniques and a laminar flow bench.

Protocol for Cryopreserved Cells

Straight after arrival, store the cryopreserved cells in liquid nitrogen, or seed them immediately.
Note: Storage at -80°C is not sufficient for cell preservation and causes irreversible cell damage.

1. Prepare the medium

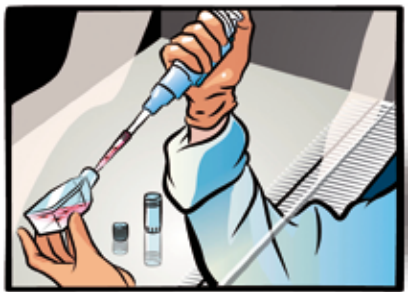
Calculate the needed culture surface area according to the plating density (see page 5). Fill the appropriate volume of PromoCell Growth Medium (at least 9 ml per vial of cells) in cell culture vessels. Place the vessels in an incubator (37°C, 5% CO_2) for 30 minutes.

2. Thaw the cells

Remove the cryovial from the liquid nitrogen container and immediately place it on dry ice - even for short transportation. Under a laminar flow bench, briefly twist the cap a quarter turn to relieve pressure, then retighten. Immerse the vial into a water bath (37°C) just up to the screw cap for 2 minutes. Ensure that no water enters the thread of the screw cap.

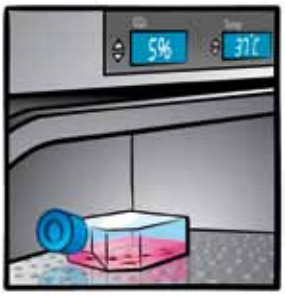
3. Disinfect the vial and seed the cells

Thoroughly rinse the cryovial with 70% ethanol under a laminar flow bench. Then, aspirate the excess ethanol from the thread area of the screw cap. Open the vial and transfer the cells to a cell culture vessel containing the prewarmed medium from step 1.



4. Incubate the cells

Place the vessel in an incubator (37°C, 5% CO_2) for cell attachment. Replace the medium after 16 – 24 hours. The cells should be subcultured, according to the subcultivation protocol (see page 4), once they have reached 70 – 90% confluency.



Start immediately after delivery.
Use aseptic techniques and a laminar flow bench.

Protocol for Proliferating Cells

1. Incubate the cells

Unpack the culture vessel, do not open the lid, and immediately place it in an incubator (37°C, 5% CO_2) for 3 hours to allow the cells to recover from the transportation.

2. Replace the transport medium

Carefully open the vessel, rinse the inner side of the lid with 70% ethanol, and let air dry. Aspirate the transport medium from the vessel. Add 10 ml of the appropriate PromoCell Cell Growth Medium.

3. Check and incubate the cells

Check the cell density. Open the lid half a turn and place the vessel in an incubator (37°C, 5% CO_2). The cells should be subcultured, according to the subcultivation protocol (see page 4), once they have reached 70 – 90% confluency.

Use aseptic techniques and a laminar flow bench.

Subcultivation Protocol

1. Prepare the reagents and wash the cells

Place the PromoCell DetachKit at room temperature for at least 30 minutes to adjust the temperature of the reagents. Carefully aspirate the medium from the culture vessel. Add 100 µl Hepes BSS Solution per cm² of vessel surface to wash the cells and agitate the vessel carefully for 15 seconds.

2. Detach the cells

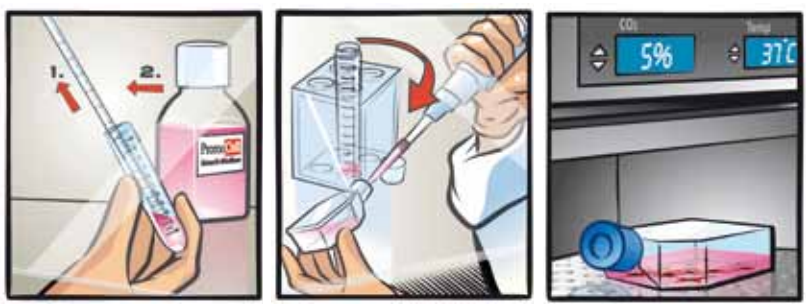
Carefully aspirate the Hepes BSS from the culture vessel. Add 100 µl Trypsin/EDTA Solution per cm² of vessel surface. Note: We recommend detaching the cells at room temperature. Close the vessel and examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells.

3. Neutralize the trypsin and harvest the cells

Add 100 µl Trypsin Neutralization Solution per cm² of vessel surface and gently agitate. Carefully aspirate the cell suspension and transfer it to a centrifugation tube. Spin down the cells for 3 minutes at 220 x g.

4. Incubate the cells

Discard the supernatant (step 1), add 1 ml of the appropriate PromoCell Cell Growth Medium (step 2), and resuspend the cells by carefully pipetting up and down. Plate the cells according to the recommended seeding density in new cell culture vessels containing PromoCell Cell Growth Medium prewarmed to 37°C. Place the vessels in an incubator (37°C, 5% CO_2).



Specifications

Product	Recommended Culture Media	Recommended Differentiation Media	Plating density
Human Mesenchymal Stem Cells from Bone Marrow (hMSC-BM)	C-28010	C-28011 C-28012 C-28013 C-28014 C-28015	4000 cells per cm^2
Human Mesenchymal Stem Cells from Umbilical Cord Matrix (hMSC-UC)	C-28010	C-28011 C-28012 C-28013 C-28014 C-28015	4000 cells per cm^2
Human Mesenchymal Stem Cells from Adipose Tissue (hMSC-AT)	C-28010	C-28011 C-28012 C-28013 C-28014 C-28015	4000 cells per cm^2

Related Products

Product	Size	Catalog Number
Mesenchymal Stem Cell Growth Medium (Ready-to-use)	500 ml	C-28010
Mesenchymal Stem Cell Adipogenic Differentiation Medium (Ready-to-use)	100 ml	C-28011
Mesenchymal Stem Cell Chondrogenic Differentiation Medium (Ready-to-use)	100 ml	C-28012
Mesenchymal Stem Cell Osteogenic Differentiation Medium (Ready-to-use)	100 ml	C-28013
Mesenchymal Stem Cell Chondrogenic Differentiation Medium w/o Inducers (Ready-to-use)	100 ml	C-28014
Mesenchymal Stem Cell Neurogenic Differentiation Medium (Ready-to-use)	100 ml	C-28015
MSC-Qualified Fetal Calf Serum	100 ml 500 ml	C-37386 C-37385
DetachKit	30 ml 125 ml 250 ml	C-41200 C-41210 C-41220
Cryo-SFM	30 ml 125 ml	C-29910 C-29912
hMSC-BM Pellet	1 million cells per pellet	C-14090
hMSC-UC Pellet	1 million cells per pellet	C-14091
hMSC-AT Pellet	1 million cells per pellet	C-14092

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