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

Shortly after isolation, all Mesenchymal Stem Cells are cryopreserved using our proprietary, defined, animal-component free, and protein-free cryopreservation medium, CryoSFM. Thawing and seeding results in passage 2. Each cryovial contains more than 500,000 viable cells after thawing. Proliferating cell cultures are made from cryopreserved cells that have been thawed and cultured for three days in our hands and shipped as growing cultures.

Quality control

We perform rigid quality control tests for each lot of Mesenchymal Stem Cells.
The cells are tested for cell morphology, proliferation potential, adherence rate, and viability. Furthermore, they are characterized by flow cytometric analysis of a comprehensive panel of markers, namely CD73/CD90/CD105 and CD14/ CD19/CD34/CD45/HLA-DR as proposed by the ISCT [1].

Differentiation assays into adipogenic, osteogenic and chrondrogenic are performed for each lot under culture conditions without antibiotics or antimycotics. In addition, all cells have been tested for the absence of HIV-1, HIV-2, HBV, HCV, HTLV-1 and HTLV-2, 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, HCV, HTLV-1 and HTLV-2, 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, cryopreserved cells should be stored in liquid nitrogen or seeded directly (see page 2). Proliferating cells must be processed immediately (see page 3).

Protocol for cryopreserved cells

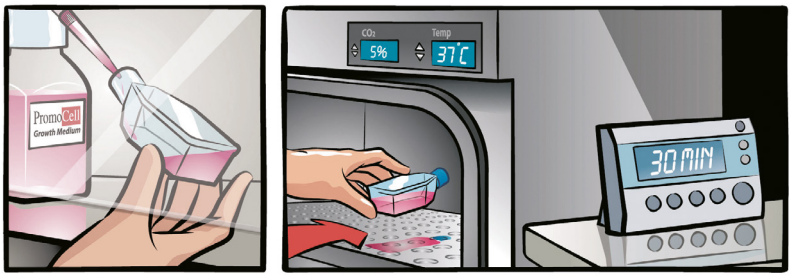
Straight after arrival, store the cryopreserved cells in liquid nitrogen or seed them immediately.

Note: For use with MSC Growth Medium XF (C-28019) fibronectin- or vitronectin-coated plasticware is needed (see 1b).

Use aseptic techniques and a laminar flow bench.

1

Prepare the medium and the culture vessel



a) For MSC Growth Medium 2
Calculate the required culture surface area according to the plating density (see page 5) and the lot-specific cell numbers stated on the certificate of analysis. 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.

b) For MSC Growth Medium XF
Calculate the required culture surface area according to the plating density (see page 5) and the lot-specific cell numbers stated on the certificate of analysis. The culture vessels must be precoated either with 1 µg/cm² human fibronectin or 0.5 µg/cm² human vitronectin according to the instruction manual of the manufacturer. Fill the appropriate volume of PromoCell Growth Medium (at least 9 ml per vial of cells) in the fibronectin- or vitronectin-coated cell culture vessels. Place the vessels in an incubator (37°C, 5% CO_2) for 30 minutes.

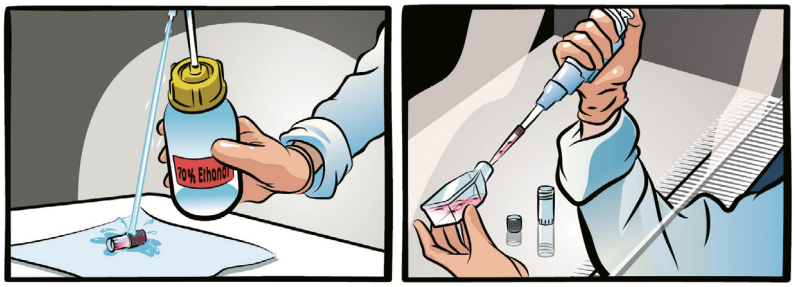
2

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 in a water bath (37°C) up to the height of 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

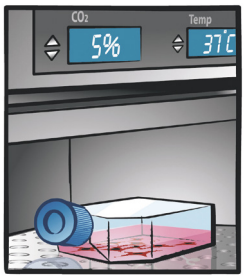


Thoroughly rinse the cryovial with 70% ethanol under a laminar flow bench. Then, aspirate the excess ethanol from the thread area of the screw cap. Open the vial and transfer the cells to a cell culture vessel containing the prewarmed medium from step 1.

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 for use with MSC Growth Medium 2. For use with serum-free MSC Growth Medium XF, replace the medium after three to four hours to avoid damaging of the cells by DMSO. Change the medium every two to three days thereafter. The cells should be subcultured according to the subcultivation protocol (see page 4) once they have reached 70–90% confluency.



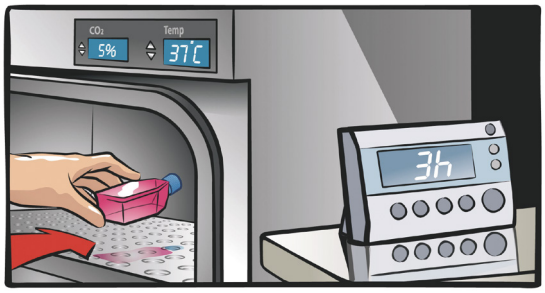
Protocol for proliferating cells

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

1

Incubate the cells

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



2

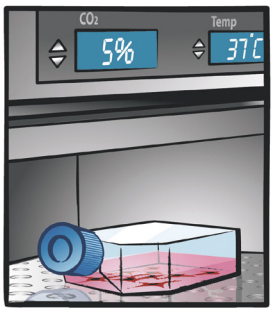
Replace the transport medium

Carefully open the vessel, rinse the inner side of the cap with 70% ethanol, and let air dry. Aspirate the transport medium (Mesenchymal Stem Cell Growth Medium 2) 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 cap half a turn and place the vessel in an incubator (37°C, 5% CO_2). Change the medium every two to three days. The cells should be subcultured, according to the subcultivation protocol (see page 4), once they have reached >70% confluency.



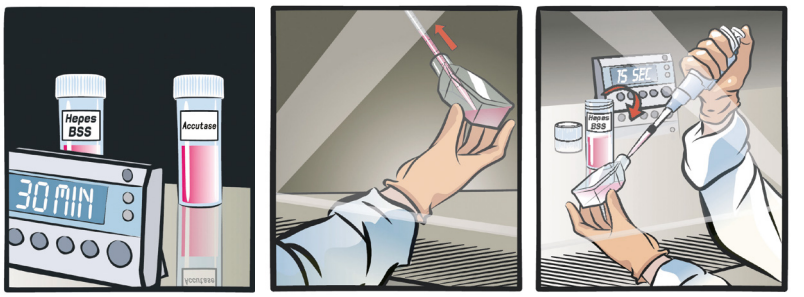
Subcultivation protocol

Use aseptic techniques and a laminar flow bench.

1

Prepare the reagents and wash the cells

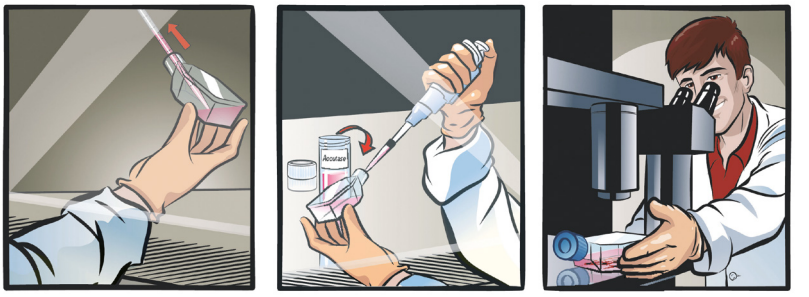
Place the Accutase-Solution at room temperature for at least 30 minutes to adjust the temperature of the reagents. Carefully aspirate the medium from the culture vessel. Add 100 µl Hepes BSS Solution per cm^2 of vessel surface to wash the cells and agitate the vessel carefully for 15 seconds.



2

Detach the cells

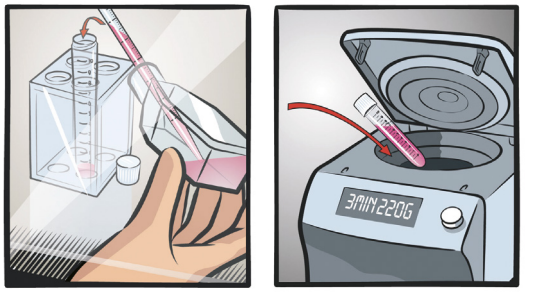
Carefully aspirate the Hepes BSS from the culture vessel. Add 100 µl Accutase-Solution per cm^2 of vessel surface and incubate for two to four minutes at room temperature. Close the vessel and examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells.



3

Harvest the cells

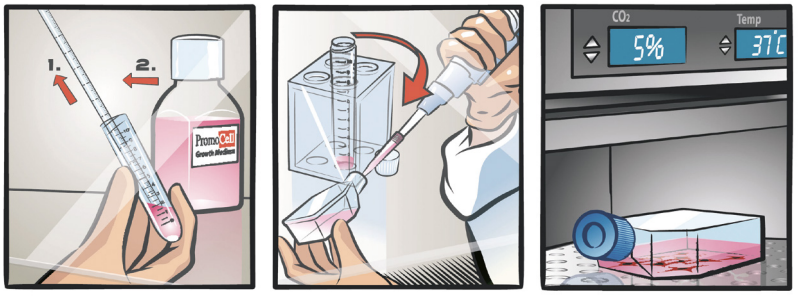
Carefully aspirate the cell suspension and transfer it to a centrifugation tube. Spin down the cells for 3 minutes at 220 x g.



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 prewarmed PromoCell Cell Growth Medium. Place the vessels in an incubator (37°C, 5% CO_2) and change the media every two to three days.



Specifications

Product	Recommended culture media	Recommended differentiation media	Plating density
Human Mesenchymal Stem Cells from Bone Marrow (hMSC-BM)	C-28009 C-28019	C-28016 C-28012 C-28013 C-28014 C-28015	4000 cells per cm²
Human Mesenchymal Stem Cells from Umbilical Cord Matrix (hMSC-UC)	C-28009 C-28019	C-28016 C-28012 C-28013 C-28014 C-28015	4000 cells per cm²
Human Mesenchymal Stem Cells from Adipose Tissue (hMSC-AT)	C-28009 C-28019	C-28016 C-28012 C-28013 C-28014 C-28015	4000 cells per cm²

Related products

Product	Size	Catalog number
Mesenchymal Stem Cell Growth Medium 2 (Ready-to-use)	500 ml	C-28009
Mesenchymal Stem Cell Growth Medium XF (Ready-to-use)	500 ml	C-28019
Mesenchymal Stem Cell Adipogenic Differentiation Medium 2 (Ready-to-use)	100 ml	C-28016
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
HEPES Buffered Saline Solution	125 ml 250 ml	C-40010 C-40020
Accutase-Solution, primary human cell culture tested	100 ml	C-41310
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
Cryo-SFM	30 ml 125 ml	C-29910 C-29912

References

[1] Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, et al., Cytother 2006, 8(4):315–7.

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