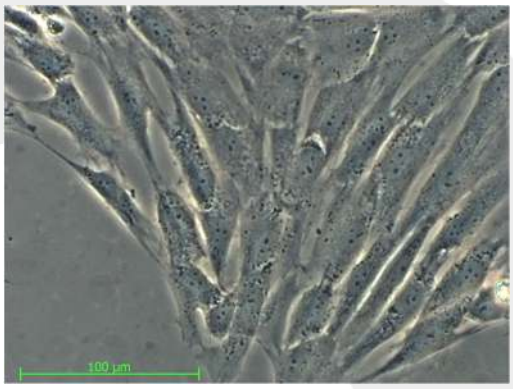
Handling Instructions

CellGenix® Human Umbilical Cord-derived Multipotent Mesenchymal Stromal Cells (hUC-MSC)

Order No.: 19401-005, 19401-010



For preclinical *ex vivo* use. Not intended for therapeutic use.

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Related products

It is recommended that CellGenix® hUC-MSC are used in conjunction with CellGenix® MSC Medium[i], CellGenix® Gelatin and CellGenix® FGF-2.

Product	Order number	Description
CellGenix® MSC Medium[i]	24803-0500	Serum-free medium developed for the optimal expansion of MSCs from umbilical cord matrix and blood
CellGenix® Gelatin	29401-0500 29401-0100	Sterile, 0.1% (w/v) ready to use solution in 0.9% sodium chloride
CellGenix® FGF-2	1008-050 1408-050 1408-010	Human recombinant FGF-2 lyophilized from a sterile-filtered solution without carrier protein

Shipment and storage

CellGenix® hUC-MSC are shipped on dry ice and should be stored in liquid nitrogen or seeded immediately upon arrival*. All other CellGenix® Products are shipped at room temperature

In accordance with IATA regulations CellGenix® hUC-MSC are assigned as **"Exempt human specimen"** for shipment. Therefore, the packaging must meet the following conditions.

The packaging must consist of three components:

- a leak-proof primary receptacle(s)
- a leak-proof secondary packaging containing absorbent material in sufficient quantity; and
- an outer packaging of adequate strength for its capacity, mass and intended use.

CellGenix® hUC-MSC are filled in cryovials. The cryovials are placed in a leak-proofed plastic tube containing absorbent material.

* **CAUTION!** Depending on your order the size of the secondary packaging tube may vary and it **may contain more than one cryovial**!
Upon arrival, promptly retrieve the cryovial(s) from the secondary packaging tube. **Place the cell containing cryovial on dry ice or in liquid nitrogen immediately!** Do not store the secondary packaging in liquid nitrogen!

Product description

Introduction

Mesenchymal stem/stromal cells (MSC) are self-renewing cells that can give rise to a variety of tissues including bone, cartilage, stromal cells and connective tissue derived from the mesoderm. The term "mesenchymal stem cell" (MSC) has originally been coined by Caplan to describe the fraction of plastic-adherent cells isolated from bone marrow[1]. Later on, the term has become to be widely used for plastic-adherent cells from other tissues including adipose tissue, umbilical cord, umbilical cord blood, amniotic fluid, and placenta. A recognized biological property of these cultured MSC is their differentiation capacity into a variety of cell types, including osteoblasts, chondroblasts, and adipocytes. However, still little information exists on how these *in vitro* observed activities regard to the biological activity of the MSC *in vivo*. In an attempt to render the terminology more precisely it has been proposed that cultured MSC should be designated "multipotent mesenchymal stromal cells", whereas the term "mesenchymal stem cell" should be reserved for the cells from primary tissues[2].

MSC hold great therapeutic promise in the field of cell-based tissue engineering and regenerative medicine. They are also being used for the prevention or treatment of Graft versus Host Disease (GvHD) after allogeneic blood stem cell transplantation, to improve engraftment of hematopoietic stem cells (HSC) *in vivo* and expansion of HSC *in vitro*. In particular, in co-culture expansion systems hUC-MSC have been shown to support cord blood cell expansion *ex vivo*[3,4,5]. In addition, UC-MSC are able to support self-renewal of human embryonic stem cells (hESC) under serum-free conditions and retain the potential of hESC to differentiate into hematopoietic cells[6,7]. Due to the early developmental origin and the lack of controversy, they are a promising source to generate human induced pluripotent stem cells (hiPSC)[8].

CellGenix® Human Umbilical Cord-derived Multipotent Mesenchymal Stromal Cells (hUC-MSC) are isolated from umbilical cord tissue by enzymatic digestion after removal of vessels to avoid endothelial contamination. They were passaged once and cryopreserved upon achieving the appropriate density. Each lot of cells originates from a single donor. All donor centers are traceable and located in Germany. All donors were healthy at the time of collection. All donors have been informed in detail about the purpose of the donation and have signed an informed consent.

Characteristics and quality control

CellGenix® hUC-MSC have been cultured serum-free before freezing using CellGenix® MSC Medium[i] containing 5 ng/ml CellGenix® FGF-2.

The freezing medium is serum-free, containing human serum albumin (HSA) and dimethyl sulfoxide (DMSO). It does not contain any antibiotics.

Each lot of cells undergoes comprehensive and rigid quality controls. The cells are tested and found to be negative for mycoplasma, bacteria, and fungi.

Viability and differentiation capacity are assayed after thawing of cryopreserved cells. The cells are assayed for their capacity to expand and to differentiate into adipogenic, osteogenic, and chondrogenic lineages. More than 95% of the cells of each lot are shown to be positive for CD73, CD90, and CD105 and negative for CD34 cell surface markers by flow-cytometry[9].

Intended use

For preclinical *ex vivo* use. Not intended for therapeutic use.

Warning

Although tested negative for HIV-1 / HBV / HCV (analyzed using maternal blood) the cells should be handled as potentially infectious material under at least Biosafety Level 1. Please review the Material Safety Data Sheet (MSDS) before handling.

Thawing and culturing the cells

General information

- Store the cells in liquid nitrogen (-196°C), or seed them directly after arrival. Storage at higher temperatures (-80°C) may cause irreversible cell damage.
- Use aseptic techniques and work in a laminar flow hood.
- Incubate cells in a humidified incubator at 37°C, 5% CO_2. Hypoxic conditions (3% O_2 are recommended but not required[10]).
- Use CellGenix® MSC Medium[i] supplemented with 5 ng/ml CellGenix® FGF-2 for growth and maintenance of CellGenix® hUC-MSC.
- Precoat cell culture plastic ware with CellGenix® Gelatin solution or equivalent coating material.
- Seed cells with a density of 2000 cells per cm^2. Do not let the cells reach confluency. Subculture cells when a density of 70% is reached.
- Use dissociating enzymes that do not need to be inactivated with serum (e.g. TrypLE™ or Accutase®)
- Resuspend cell pellets by careful pipetting. Do not vortex the cells.

Preparation of coated cell culture plastic ware

- Coat culture dish surface with at least 100 µl CellGenix® Gelatin solution per cm^2.
- Incubate for 30 min. at room temperature.
- Remove the gelatin solution and discard.
- Add cells to the dish immediately. Do not allow the dish to dry before adding the cells. It may be necessary to determine optimal conditions for attachment for each application and equipment used.

Thawing of cells

Do not thaw cells until all required materials and equipment are on hand. Cryopreserved cells are extremely fragile and require gentle handling and immediate placement into pre-warmed culture medium.

1. Prepare a 15 ml conical centrifugation tube containing 10 ml pre-warmed CellGenix® MSC Medium[i].
2. Remove cells from liquid nitrogen (transport on dry ice if necessary).
3. Place vial of cells in 37°C water bath ensuring the cap is tight. Agitate gently until only small pieces of ice remain.
4. Immediately disinfect the vial with 70% ethanol.
5. Transfer cells into medium containing centrifugation tube and centrifuge at 300 x g for 5 min.
6. Aspirate supernatant and carefully resuspend cells in CellGenix® MSC Medium[i] containing 5 ng/ml freshly added CellGenix® FGF-2. Seed the cells in a cell culture dish coated with CellGenix® Gelatin with a density of 2000 to 4000 cells per cm^2.

Incubate cells at 37°C in a 5% CO_2, 3% O_2 humidified incubator.

Subculturing cells

Prepare all required materials and equipment before removing the cells from the incubator. Pre-warm all solutions and media which come in contact with the cells.

1. Aspirate medium and wash cells with Phosphate Buffered Saline (PBS Ca^{++}/Mg^{++} free) once.
2. Submerge cells with a sufficient volume of Accutase® or TrypLE™ solution. Incubate for 5 min. at 37°C. If necessary, detach cells by gently tapping the side of the cell culture vessel.
3. Add cell culture medium to dilute the enzyme solution (add at least twice the volume of the enzyme solution used).
4. Transfer cell suspension to a centrifugation tube and spin down the cells for 5 min. at 300 x g.
5. Discard supernatant and resuspend cells in CellGenix® MSC Medium[i] containing 5 ng/ml freshly added CellGenix® FGF-2 by carefully pipetting up and down. Determine cell concentration.
6. Seed the cell suspension in new, freshly CellGenix® Gelatin coated cell culture dishes with a density of 2000 cells per cm^2.

Incubate cells at 37°C in a 5% CO_2, 3% O_2 humidified incubator. Subculture again when a cell density of 70% is reached. Subculturing is usually required twice a week.

References

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2. Horwitz *et al.* (2005) Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. Cytotherapy Vol. 7, 393-395.
3. Friedman *et al.* (2007) Umbilical cord mesenchymal stem cells: adjuvantsfor human cell transplantation. Biol Blood Marrow Transplant Vol. 13, 1477-1486.
4. Magin *et al.* (2009) Primary cells as feeder cells for coculture expansion of human hematopoietic stem cells from umbilical cord blood - a comparative study. Stem Cells Dev Vol. 18, 173-186.
5. Robinson *et al.* (2011) Mesenchymal stem cells in *ex vivo* cord blood expansion. Best Pract Res Clin Haematol Vol 24,83-92.
6. Cho *et al.* (2010) Human feeder layer system derived from umbilical cord stromal cells for human embryonic stem cells. Fertility and Sterility Vol. 93, 2525-2531.
7. Hiroyama *et al.* (2008) Human umbilical cord-derived cells can often serve as feeder cells to maintain primate embryonic stem cells in a state capable of producing hematopoietic cells. Cell Biol Int Vol. 32, 1-7.
8. Cai *et al.* (2010) Generation of human induced pluripotent stem cells from umbilical cord matrix and amniotic membrane mesenchymal cells. J Biol Chem Vol. 285, 11227-11234.
9. Dominici *et al.* (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy Vol. 8, 315-317.
10. Nekanti *et al.* (2010) Increased proliferation and analysis of differential gene expression in human Wharton's Jelly-derived mesenchymal stromal cells und hypoxia. Int J Biol Sci Vol. 6, 499-512.

[i] CellGenix® MSC Medium is a non-stock item, please request at an early stage

Technical support

- Lot specific certificates of analysis are part of each delivery.
- Material safety data sheets (MSDS) are available on request.

Please contact us for more information or technical assistance

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For data sheet and related products please visit the CellGenix Web site at cellcenix.com.

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