Last updated: November 25, 2019

CellSource

Adipose-Derived Mesenchymal Stem Cells

Product Information

Adipose-Derived Mesenchymal Stem Cells (ADSCs) are multipotent stem cells capable of differentiating into a diverse range of cell types, including adipocytes, osteocytes, and chondrocytes. In addition to their multipotent properties, ADSCs are also known for their abilities to promote wound healing, to stimulate angiogenesis, and to modulate immune responses. These therapeutic potentials of ADSCs have increasingly been recognized in studies of various diseases such as myocardial infarction, stroke, and liver failure.

One tube of Adipose-Derived Mesenchymal Stem Cells contains a minimum of 5×10^5 cells.

Characteristics of ADSCs

Tissue of origin: adipose
Morphology: spindle-shaped, fibroblast-like
Growth properties: adherent

Safety Precaution

Wear protective gloves and a face mask when handling cryotubes. Liquid nitrogen may seep into the tube during storage and evaporate rapidly during the thawing process. This rapid liquid-to-gas expansion may cause the tube to explode and create flying debris.
Also, the provided cells should be treated in the same way as those known to pose serious health risks such as HIV-infected materials. Even if the results obtained from a virus detection assay are negative, the existence of infectious agents/genomes cannot be completely denied.

Unpacking and Storage Procedures

1. Remove cryotubes from the dry ice and inspect them for deformation, breakage, and leakage.
2. Immediately transfer the cryotubes into a liquid nitrogen storage tank. Keep the tubes in the vapor phase of liquid nitrogen until ready for use.

Recommended Growth Medium

Mesenchymal Stem Cell Growth Medium 2 (Ready-to-use) (Promo Cell, Cat. No. C-28009)
*Follow manufacturer's instructions to prepare the complete growth medium.

CellSource

Thawing Cryopreserved Cells for Initial Culturing

1. Add an appropriate amount of complete growth medium into cell culture flask(s) (15 mL per 75 cm^2 of surface area). Place the flask(s) in a 37°C incubator equilibrated to 5% CO_2 atmosphere.
2. Remove the cryotube out of the liquid nitrogen storage tank. While working in a clean bench, loosen the cap slightly to relieve the potential pressure buildup due to liquid nitrogen leaking into the tube during storage. Tighten the cap before taking the tube out of the bench.
3. Rapidly warm the cryotube with gentle agitation in a 37°C water bath. Do not leave the cells in the water bath for more than two minutes. Before bringing the tube into the clean bench, make sure to dry the surface of the tube with a paper towel and to wipe it off with 70% ethanol. All operations from here should be carried out under aseptic conditions.
4. Transfer the cells (tube contents) to 5 mL of pre-warmed growth medium in a centrifuge tube.
5. Centrifuge the cells at 600 x g for 5 minutes.
6. Discard the supernatant. Resuspend the cell pellet with 5 mL of complete growth medium, and then count the cells.
7. Seed the culture flask(s) prepared in step 1 at a cell density of 5,000-6,000 cells/cm^2. Gently shake the flask(s) to spread the cells throughout the flask(s).
8. Place the seeded culture flask(s) in the 37°C incubator equilibrated to 5% CO_2 atmosphere.

Cell Maintenance (Medium Change)

1. 72 ~ 96 hours after seeding, remove the flasks from the incubator and view the cells under a microscope to estimate cellular confluence. If cultures have already reached 70% to 80% confluence, it is time to subculture, so proceed to subculturing as described in the next section. If cultures are not ready for subculturing, perform the following steps.
2. Warm the complete growth medium in the 37°C incubator equilibrated to 5% CO_2 atmosphere (15 mL per 75 cm^2 of surface area).
3. Discard the spent medium carefully so as not to disturb the cell monolayer.
4. Add the pre-warmed complete growth medium. Return the flasks to the incubator.
5. After 72 hours, view each flask under the microscope to estimate cellular confluence. If cultures have reached 70% to 80% confluence, proceed to subculturing. If not, repeat steps 2-4 as described above.

CellSource

Subculturing

*Passage the cells when cultures have reached approximately 70% to 80% confluence.

*Solution volumes are given for culture flasks with 75 cm^2 of surface area.

1. Add 15 mL of complete growth medium into cell culture flask(s.) Place the flask(s) in the 37°C incubator equilibrated to 5% CO_2 atmosphere for about 30 minutes.
2. Discard the spent medium carefully so as not to disturb the cell monolayer.
3. Rinse the cell layer twice with 5 mL of D-PBS (calcium-free, magnesium-free).
4. Add 3 mL of pre-warmed trypsin-EDTA solution (Biological Industries、Cat. No. 03-079-1A) to cover the cell layer. Incubate the cells with trypsin at 37°C for 5 minutes.
5. Observe the cells under the microscope. If most cells start to round up and detach from the flask surface, gently tap the flask from sides to ensure the complete detachment of cells. If not, leave the cells at 37°C for a few more minutes to promote cellular detachment.
6. Quickly add to the flask an equal volume of PBS-diluted (1:50) Soybean Trypsin Inhibitor (Biological Industries, Cat. No. 03-048-1). Gently pipette the solutions to ensure that trypsin-EDTA has been neutralized.
7. Transfer the dissociated cells to a sterile centrifuge tube and centrifuge at 600 x g for 5 minutes.
8. Discard the supernatant. Resuspend the cells in 10 mL of complete growth medium, and then count the cells.
9. Seed new culture flask(s) at a cell density of 5,000-6,000 cells/cm^2.
10. Place the seeded flask(s) in the 37°C incubator equilibrated to 5% CO_2 atmosphere.

Cryopreservation

*Cryopreserve the cells when cultures have reached approximately 70% to 80% confluence.

[Note] Do not allow cells to become fully confluent. It is recommended that the culture medium be changed the day before cryopreservation.

1. Add the appropriate volume of pre-culture medium into the tube(s). Place the medium in the 37°C incubator equilibrated to 5% CO_2 atmosphere for about 30 minutes before use.
2. Discard the spent medium carefully so as not to disturb the cell monolayer.
3. Rinse the cell layer twice with 5 mL of D-PBS (calcium-free, magnesium-free).
4. Add 3 mL of pre-warmed trypsin-EDTA solution (Biological Industries、Cat. No. 03-079-1A) to cover the cell layer. Incubate the cells with trypsin at 37°C for 5 minutes.
5. Observe the cells under the microscope. If most cells start to round up and detach from the flask surface, gently tap the flask from sides to ensure the complete detachment of cells. If not, leave the cells at 37°C for a few more minutes to promote cellular detachment.

6. Quickly add to the flask an equal volume of PBS-diluted (1:50) Soybean Trypsin Inhibitor (Biological Industries, Cat. No. 03-048-1). Gently pipette the solutions to ensure that trypsin-EDTA has been neutralized.
7. Transfer the dissociated cells to a sterile centrifuge tube and centrifuge at 600 x g for 5 minutes.
8. Discard the supernatant. Resuspend the cells in 10 mL of complete growth medium, and then count the cells.
9. Centrifuge the cells at 600 x g for 5 minutes.
10. Discard the supernatant. Resuspend the cells in STEM-CELLBANKER® DMSO Free GMP grade (Zenoaq, Cat. No. ZR645) at a cell concentration of $5x10^5$ - $5x10^6$ cells/mL.
11. Aliquot 1 mL of resuspended cells into each cryotube. Place the cryotubes in a cryopreservation vessel such as BICELL (Nihon Freezer). Store the vessel at -80°C overnight to freeze the cells gradually.
12. Transfer the cryotubes to a liquid nitrogen storage tank, preferably the vapor phase of liquid nitrogen.

Recommended Medium for Adipogenesis

- Mesenchymal Stem Cell Growth Medium 2 (Ready-to-use) (Promo Cell, Cat. No. C-28009)
- Mesenchymal Stem Cell Adipogenic Differentiation Medium 2 (Ready-to-use) (Promo Cell, Cat. No. C-28016)

*Follow manufacturer's instructions to prepare the media and to induce adipogenesis.

Recommended Medium for Chondrogenesis

- Mesenchymal Stem Cell Growth Medium 2 (Ready-to-use) (Promo Cell, Cat. No. C-28009)
- Mesenchymal Stem Cell Chondrogenic Differentiation Medium (Ready-to-use) (Promo Cell, Cat. No. C-28012)

*Follow manufacturer's instructions to prepare the media and to induce chondrogenesis.

Recommended Medium for Osteogenesis

- Mesenchymal Stem Cell Growth Medium 2 (Ready-to-use) (Promo Cell, Cat. No. C-28009)
- Mesenchymal Stem Cell Osteogenic Differentiation Medium (Ready-to-use) (Promo Cell, Cat. No. C-28013)

*Follow manufacturer's instructions to prepare the media and to induce osteogenesis.

Last updated: November 25, 2019

Biosafety Level: 1

Appropriate safety procedures should always be used with this material.

Manufactured by CellSource Co., Ltd.

Distributed by Summit Pharmaceuticals International Corporation

Contact

Summit Pharmaceuticals International Corporation

Biology Group, Discovery & Alliance Coordination Dept.
Sumitomo Corporation Takebashi BLD. 12F
1-2-2, Hitotsubashi, Chiyoda-ku, Tokyo 100-0003, Japan
Tel: +81-3-5220-1561
E-mail: alliance@summitpharma.co.jp