Human iPSC-Derived Mesenchymal Progenitor Cells

Catalog #200-0781 1 x 10^6 cells

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Product Description

Human induced pluripotent stem cell (iPSC)-Derived Mesenchymal Progenitor Cells (MPCs) are highly pure multipotent progenitor cells derived and manufactured from Healthy Control Human iPSC Line, Female, SCTi003-A (Catalog #200-0511), using STEMdiff™ Mesenchymal Progenitor Kit (Catalog #05240). MPCs express high levels of mesenchymal cell markers such as CD73, CD90, and CD105.

MPCs are capable of long-term expansion when cultured with MesenCult™-ACF Plus Medium (Catalog #05445; also included in MesenCult™-ACF Plus Culture Kit #05448) and cryopreserved using MesenCult™-ACF Freezing Medium (Catalog #05490), allowing for workflow flexibility.

MPCs can be reliably differentiated into cells of mesenchymal lineage, such as adipocytes [MesenCult™ Adipogenic Differentiation Kit (Human; Catalog #05412)], osteoblasts [MesenCult™ Osteogenic Differentiation Kit (Human; Catalog #05465)], and chondrocytes (MesenCult™-ACF Chondrogenic Differentiation Kit (Catalog #05455)].

MPCs, as well as the adipogenic, osteogenic, and chondrogenic cells generated from the MPCs, can be used for studying regenerative medicine development, drug discovery, and disease modeling.

Cells were obtained using Institutional Review Board (IRB)-approved consent forms and protocols.

Stability and Storage

Cells are frozen in a cryopreservation medium containing dimethyl sulfoxide (DMSO). Product stable at -135°C or colder for 12 months from date of receipt. Thawed samples must be used immediately.

Precautions

Cell Screening: iPSC master cell banks are screened for AAV2, BK virus, Epstein-Barr Virus, Hepatitis A, Hepatitis B, Hepatitis C, Herpes Simplex 1 and 2, Herpes Virus Type 6, 7, and 8, HIV-1, HIV-2, HPV-16, HPV-18, Human Adenovirus, Human Cytomegalovirus, Human Foamy Virus, Human T-Lymphotropic Virus, John Cunningham Virus, LCMV, Parvovirus B19, Sarbecovirus (SARS Virus), Seoul Virus, Corynebacterium Bovis, and Mycoplasma (Human Comprehensive CLEAR Panel) by PCR. As testing cannot completely guarantee that the donor was virus-free, THIS PRODUCT SHOULD BE TREATED AS POTENTIALLY INFECTIOUS and only used following appropriate handling precautions such as those described in biological safety level 2.

Storage of frozen cell products in the vapor phase of a liquid nitrogen storage tank is recommended. Storage in the liquid phase can result in cross-contamination if the vial breaks or is not sealed properly. Storage in the liquid phase also increases the potential for liquid nitrogen to penetrate the vial and cause it to explode when removed from storage. Use of a face shield is required as a safety precaution when transferring cells from one container to another. When handling this product, do not use sharps such as needles and syringes.

STEMCELL cannot guarantee the biological function or any other properties associated with performance of cells in a researcher's individual assay or culture systems. STEMCELL assures the cells will meet the specifications only when assessed immediately after thawing by our test methods.

FOR IN VITRO RESEARCH USE ONLY. NOT APPROVED FOR DIAGNOSTIC, THERAPEUTIC, OR CLINICAL APPLICATIONS. NOT APPROVED FOR HUMAN OR VETERINARY USE IN VIVO.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
0.5 mL screw cap polypropylene tubes	e.g. Sarstedt 72.785.005
Animal Component-Free Cell Dissociation Kit • ACF Enzymatic Dissociation Solution • ACF Enzyme Inhibition Solution	05426
Cell Scraper, Lifter Blade OR Cell Lifter, Double End	200-0594 OR 200-0596
Costar® 6-Well Flat-Bottom Plate, Tissue Culture-Treated	38015
D-PBS (Without Ca++ and Mg++)	37350
Hausser Scientific™ Bright-Line Hemocytometer	100-1181
L-Glutamine	07100
MesenCult™-ACF Freezing Medium	05490
MesenCult™-ACF Plus Culture Kit • MesenCult™-ACF Plus Medium • MesenCult™-ACF Plus 500X Supplement • Animal Component-Free Cell Attachment Substrate	05448
Polypropylene conical tubes, 15 mL and 50 mL	e.g. 38009 and 38010
T-75 cm^2 flask, tissue culture-treated	e.g. 200-0501
Trypan Blue	07050

Preparation of Reagents and Materials

A. COATING CULTUREWARE WITH ANIMAL COMPONENT-FREE CELL ATTACHMENT SUBSTRATE

Use sterile technique when coating cultureware with Animal Component-Free Cell Attachment Substrate.

NOTE: Use only tissue culture-treated cultureware.

1. Dilute Animal Component-Free Cell Attachment Substrate 1 in 300 in D-PBS (Without Ca++ and Mg++).
2. Gently mix the diluted substrate solution. Do not vortex.
3. Immediately use the diluted substrate solution to coat cultureware. Refer to Table 1 for recommended coating volumes.
4. Gently tilt the cultureware to spread the diluted substrate solution evenly across the surface.
5. Incubate at room temperature (15 - 25°C) for at least 2 hours before use. Do not let the diluted substrate solution evaporate.

   NOTE: If not used immediately, the cultureware must be sealed to prevent evaporation of the diluted substrate solution (e.g. with Parafilm®). Sealed cultureware can be stored at 2 - 8°C for up to 3 days after coating. Allow stored coated cultureware to come to room temperature for 30 minutes before proceeding to step 6.
6. Gently tilt the cultureware onto one side and allow excess diluted substrate solution to collect at the edge. Remove the excess solution using a serological pipette or by aspiration. Ensure that the coated surface is not scratched.
7. Wash cultureware once, using D-PBS (e.g. use 12 mL if using a T-75 cm^2 flask).
8. Aspirate wash solution when MPCs are ready to be plated.

Table 1. Recommended Volumes for Coating Cultureware with Diluted Animal Component-Free Cell Attachment Substrate

CULTUREWARE	VOLUME OF DILUTED ANIMAL COMPONENT-FREE CELL ATTACHMENT SUBSTRATE SOLUTION
6-well plate	1 mL/well
T-25 cm^2 flask	2.5 mL/flask
T-75 cm^2 flask	6 mL/flask

B. COMPLETE MESENCULT™-ACF PLUS MEDIUM

Use sterile technique to prepare complete MesenCult™-ACF Plus Medium (MesenCult™-ACF Plus Medium + MesenCult™-ACF Plus 500X Supplement + L-Glutamine). The following example is for preparing 500 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw MesenCult™-ACF Plus 500X Supplement on ice for 1 - 2 hours or overnight at 2 - 8°C. Mix thoroughly.

   NOTE: Once thawed, use immediately or aliquot and store at -20°C. For aliquoting, use 0.5 mL screw cap polypropylene tubes. Do not exceed the shelf life of the supplement. After thawing the aliquots, use immediately. **Do not re-freeze.**
2. Add 1 mL of MesenCult™-ACF Plus 500X Supplement to 500 mL of MesenCult™-ACF Plus Medium. Mix gently and thoroughly.
3. Add L-Glutamine to a final concentration of 2 mM. Mix thoroughly.

   NOTE: If not used immediately, store complete MesenCult™-ACF Plus Medium **at 2 - 8°C for up to 1 week**. Do not exceed the shelf life of the individual components. **Do not store complete MesenCult™-ACF Plus Medium at -20°C.**

Directions for Use

A. THAWING AND PLATING MESENCHYMAL PROGENITOR CELLS

Generally, 1 x 10^6 of Human iPSC-Derived Mesenchymal Progenitor Cells is enough to seed three T-75 cm^2 flasks at the higher range of recommended seeding densities or six T-75 cm^2 flasks at the lower range of recommended seeding densities.

NOTE: The following instructions are for seeding cells into coated T-75 cm^2 flasks. If using other cultureware, adjust volumes accordingly.

NOTE: Pre-coating of the cultureware is generally required. Cultureware should be prepared in advance as described above (see Preparation of Reagents and Materials, section A) and brought to room temperature (15 - 25°C) for at least 30 minutes prior to use.

1. Warm complete MesenCult™-ACF Plus Medium to 37°C (see Preparation of Reagents and Materials, section B).
2. Wipe the outside of the vial of cells with 70% ethanol or isopropanol.
3. In a biosafety cabinet, twist the cap a quarter-turn to relieve internal pressure and then retighten.
4. Quickly thaw cells in a 37°C water bath by gently shaking the vial. Remove the vial when a small frozen cell pellet remains. Do not vortex cells.
5. Remove the cryovial from the water bath and wipe the outside of the vial with 70% ethanol or isopropanol.

   NOTE: It is important to work quickly in the following steps to ensure high cell viability and recovery.
6. Measure and record the total volume of the cell suspension using a 2 mL serological pipette.
7. Remove a 20 µL aliquot of cells for pre-wash counting. If using Trypan Blue to assess viability, we suggest adding a minimum of 20 µL of medium and recording the volume of medium added.
8. Transfer cells from the cryovial to a 15 mL polypropylene conical tube containing 10 mL of warm complete MesenCult™-ACF Plus Medium. Mix gently.
9. Rinse the vial with 1 mL of warm complete MesenCult™-ACF Plus Medium and add it to the cells in the 15 mL polypropylene conical tube from Step 8, while gently swirling the 15 mL tube.
10. Centrifuge cells at 300 x g for 5 minutes at room temperature.
11. Carefully remove the supernatant with a pipette, leaving a small amount of medium to ensure the cell pellet is not disturbed. Resuspend the cell pellet by gently flicking the tube.
12. Add 2 mL of culture medium to the tube. Mix gently.
13. Count viable cells using Trypan Blue and a hemocytometer.
14. Plate cells into pre-coated cultureware containing complete MesenCult™-ACF Plus Medium. Refer to Table 2 for recommended volumes.

    NOTE: Recommended seeding density is 2 - 4 x 10^3 cells/cm^2. Move the cultureware in several quick, short, back-and-forth, and side-to-side motions to evenly distribute the MPCs across the surface. Place the cultureware in a 37°C incubator and repeat several quick, short, back-and-forth, and side-to-side motions to evenly distribute the MPCs across the surface.
15. Incubate cells at 37°C for approximately 3 - 6 days. Passage cells when they are approximately 80% confluent.

    NOTE: Half-medium changes are only required if cells start to detach.

B. EXPANSION OF MESENCHYMAL PROGENITOR CELLS

The following protocol is for expansion in T-75 cm^2 flask. If using other cultureware, refer to Table 2 for adjusted cell numbers and volumes.

NOTE: Only use tissue culture-treated cultureware.

1. Warm ACF Enzymatic Dissociation Solution, ACF Enzyme Inhibition Solution, and complete MesenCult™-ACF Plus Medium to room temperature (15 - 25°C).

   NOTE: Do not incubate at 37°C.
2. Wash the flask once with 12 mL of D-PBS (Without Ca++ and Mg++). Discard the wash.
3. Add 6 mL of ACF Enzymatic Dissociation Solution. Incubate at 37°C for 3 - 6 minutes. Tap the flask to detach cells. If less than 80% of cells have detached, incubate at 37°C for an additional 1 - 2 minutes and tap the flask again. Do not exceed 7 minutes of incubation.

   NOTE: Regardless of whether the cells detach, proceed to the next step.
4. Add 6 mL of ACF Enzyme Inhibition Solution and collect cells in a polypropylene conical tube.
5. Wash the flask with 12 mL of complete MesenCult™-ACF Plus Medium and check the flask under a microscope. If > 20% of the cells remain attached, use a cell scraper/lifter to gently detach cells. Transfer to the polypropylene conical tube from step 4.
6. Centrifuge the tube at 300 x g for 8 minutes.
7. Discard the supernatant. Resuspend the cell pellet in 2 mL complete MesenCult™-ACF Plus Medium.
8. Count viable cells using Trypan Blue and a hemocytometer.
9. Plate cells into pre-coated cultureware (e.g. T-75 cm^2 flask or 6-well plate).

   Note: Recommended cell plating density is 2 - 4 x 10^3 cells/cm^2. Move the cultureware in several quick, short, back-and-forth, and side-to-side motions to evenly distribute the MPCs across the surface. Place the cultureware in a 37°C incubator and repeat several quick, short, back-and-forth, and side-to-side motions to evenly distribute the MPCs across the surface.
10. Incubate cells at 37°C for approximately 3 - 6 days. Passage cells when they are approximately 80% confluent.

    NOTE: Half-medium changes are only required if cells start to detach. iPSC-Derived MPCs are cryopreserved at approximately passage 5 after mesenchymal induction. It is recommended to record the passage number of the MPCs and periodically assess the cells (see section D). For best efficiency in downstream differentiation to different cell types, follow instructions in the applicable Product Information Sheets (Table 4).

Table 2. Recommended Volumes and Cell Plating Densities for Passaging

VOLUME OF SOLUTIONS AND CELL NUMBER	6-WELL PLATE	T-25 cm^2 FLASK	T-75 cm^2 FLASK
ACF Enzymatic Dissociation Solution	1 mL/well	2.5 mL/flask	6 mL/flask
ACF Enzymatic Inhibition Solution	1 mL/well	2.5 mL/flask	6 mL/flask
Complete MesenCult™-ACF Plus Medium	2 mL/well	5 mL/flask	12 mL/flask
D-PBS Wash	2.5 mL/well	5 mL/flask	12 mL/flask
Recommended Cell Numbers	2 - 4 x 10^4	5 - 10 x 10^4	1.5 - 3 x 10^5

C. CRYOPRESERVATION OF MESENCHYMAL PROGENITOR CELLS

1. Wipe the outside of MesenCult™-ACF Freezing Medium container with 70% ethanol or isopropanol before opening.
2. Prepare a single-cell suspension of human MPCs using Animal Component-Free Cell Dissociation Kit (see steps 1 - 5 in section B above) and centrifuge cells at 300 x g for 8 minutes to obtain a cell pellet.
3. Carefully remove the supernatant with a pipette, leaving a small amount of medium to ensure the cell pellet is not disturbed. Resuspend the cell pellet by gently flicking the tube.
4. Count viable cells using Trypan Blue and a hemocytometer.
5. Add cold (2 - 8°C) MesenCult™-ACF Freezing Medium to obtain a cell suspension of 1 x 10^6 cells/mL and mix thoroughly.
6. Transfer 1 mL of the cell suspension into each cryovial.
7. Freeze cells using a standard slow rate-controlled cooling protocol (approximately -1°C/minute) or an isopropanol freezing container and store at -135°C (liquid nitrogen).

   NOTE: Long-term storage at -80°C is not recommended.

D. ASSESSMENT OF MESENCHYMAL PROGENITOR CELLS

Assessment of MPCs can be verified by flow cytometry after labeling with fluorochrome-conjugated antibodies. Refer to Table 3 for recommended antibody clones and expected expression levels for assessing mesenchymal progenitor cells by immunocytochemistry or flow cytometry. Most cells (i.e. ≥ 90%) express CD73, CD105, and CD146 and do not express hematopoietic (CD45, CD34) or endothelial (CD144, CD31) markers. The absence of undifferentiated cells can be confirmed by flow cytometry after labeling with fluorochrome-conjugated anti-OCT4 and anti-TRA-1-60.

Table 3. Recommended Clones and Expected Expression Levels for Mesenchymal Progenitor Assessment

ANTIBODY TARGET	RECOMMENDED ANTIBODY FOR FLOW CYTOMETRY	EXPECTED EXPRESSION
CD73	Anti-Human CD73 (Ecto-5'-Nucleotidase) Antibody, Clone AD2 (Catalog #60044)*	≥ 90%
CD90	Anti-Human CD90 Antibody, Clone 5E10 (Catalog #60045)*	≥ 90%
CD105	Anti-Human CD105 (Endoglin) Antibody, Clone SN6h (Catalog #100-0243)*	≥ 90%
CD45	Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018)*	≤ 1%
CD34	Anti-Human CD34 Antibody, Clone 581 (Catalog #60013) OR Clone 8G12 (Catalog #60121)	≤ 1%
OCT4	Anti-Mouse OCT4 (OCT3) Antibody, Clone 40 (Catalog #60059) OR Clone 3A2A20 (Catalog #60093)	≤ 1%
TRA-1-60	Anti-Human TRA-1-60 Antibody, Clone TRA-1-60R (Catalog #60064)	≤ 1%

*This antibody is also available as part of MSC Characterization Antibody Panel (Catalog #100-0354)

E. PASSAGING MESENCHYMAL PROGENITOR CELLS FOR DOWNSTREAM DIFFERENTIATION

MPCs can be further differentiated into adipocytes, osteoblasts, and chondrocytes. For best results, culture MPCs for at least one passage after thaw before starting downstream differentiation protocols. Refer to Table 4 for recommended products. For instructions on differentiation into specific cell types, refer to the Product Information Sheets of recommended products, available at www.stemcell.com, or contact us to request a copy.

Table 4. Cell Types and Products Applicable for Downstream Differentiation of MPCs

CELL TYPE	PRODUCTS
Adipocytes	MesenCult™ Adipogenic Differentiation Kit (Human; Catalog #05412)
Chondrocytes	MesenCult™-ACF Chondrogenic Differentiation Kit (Catalog #05455)
Osteoblasts	MesenCult™ Osteogenic Differentiation Kit (Human; Catalog #05465)

Notes and Tips

The use of polypropylene tubes (e.g. Catalog #38009 and #38010) during subculture will help prevent the MSCs from sticking to the tubes.

Related Products

For related products, including specialized cell culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com/hPSCNCworkflow, or contact us at techsupport@stemcell.com.