STEMdiff™ Mesenchymal Progenitor Kit

Defined culture kit for derivation and expansion of mesenchymal progenitor cells

Catalog #05240 1 Kit

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Product Description

STEMdiff™ Mesenchymal Progenitor Kit is a defined culture kit consisting of animal component-free (ACF) induction medium, expansion medium, and attachment substrate. It is optimized for the derivation of cells with mesenchymal progenitor cell (MPC)-like properties from human embryonic stem cells (hESCs) or induced pluripotent stem cells (hiPSCs). This kit provides a complete workflow of defined reagents for derivation and expansion of hESC- or hiPSC-derived MPCs.

For animal component-free and optimized cryopreservation, MesenCult™-ACF Freezing Medium (Catalog #05490) is recommended for human MSCs previously cultured in MesenCult™ media, including MesenCult™-ACF Plus. For a complete list of related products, including differentiation media available, visit www.stemcell.com, or contact us at techsupport@stemcell.com.

Product Information

The following components are sold as a complete kit (Catalog #05240) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMdiff™-ACF Mesenchymal Induction Medium	05241	100 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
MesenCult™-ACF Plus Medium	05446	500 mL	Store at 2 - 8°C.	Stable for 2 years from date of manufacture (MFG) on label.
MesenCult™-ACF Plus 500X Supplement	05447	1 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
Animal Component-Free Cell Attachment Substrate	07130	1 mL	Store at 2 - 8°C.	Stable until expiry (EXP) date on label.

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, Scraper Blade OR Cell Lifter, Double End	200-0594 OR 200-0596
Costar® 6-Well Flat-Bottom Plate, Tissue Culture-Treated	38015
Corning® Matrigel® hESC-Qualified Matrix OR Vitronectin XF™	Corning 354277 OR 07180
DMEM/F-12 with 15 mM HEPES	36254
D-PBS (Without Ca++ and Mg++)	37350
Falcon® 6-Well Flat-Bottom Plate, Tissue Culture-Treated	38016
Gentle Cell Dissociation Reagent	100-0485
Hausser Scientific™ Bright-Line Hemocytometer	100-1181
L-Glutamine	07100

mTeSR™1 OR mTeSR™ Plus OR TeSR™-E8™	85850 OR 05825 OR 05990
Polypropylene conical tubes, 15 mL and 50 mL	e.g. 38009 and 38010
Serological pipettes, 1 mL	e.g. 38001
Trypan Blue	07050
Y-27632 (Dihydrochloride)	72302

Preparation of Media

STEMdiff™-ACF Mesenchymal Induction Medium

Thaw STEMdiff™-ACF Mesenchymal Induction Medium at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.

NOTE: Do not filter STEMdiff™-ACF Mesenchymal Induction Medium. Once thawed, use immediately or store at 2 - 8°C for up to 1 month. Alternatively, aliquot into polypropylene or polyethylene terephthalate (PETE) tubes or bottles and store at -20°C. After thawing aliquots, use immediately or store at 2 - 8°C for up to 2 weeks. Do not re-aliquot into additional tubes or bottles.

Complete MesenCult™-ACF Plus Medium

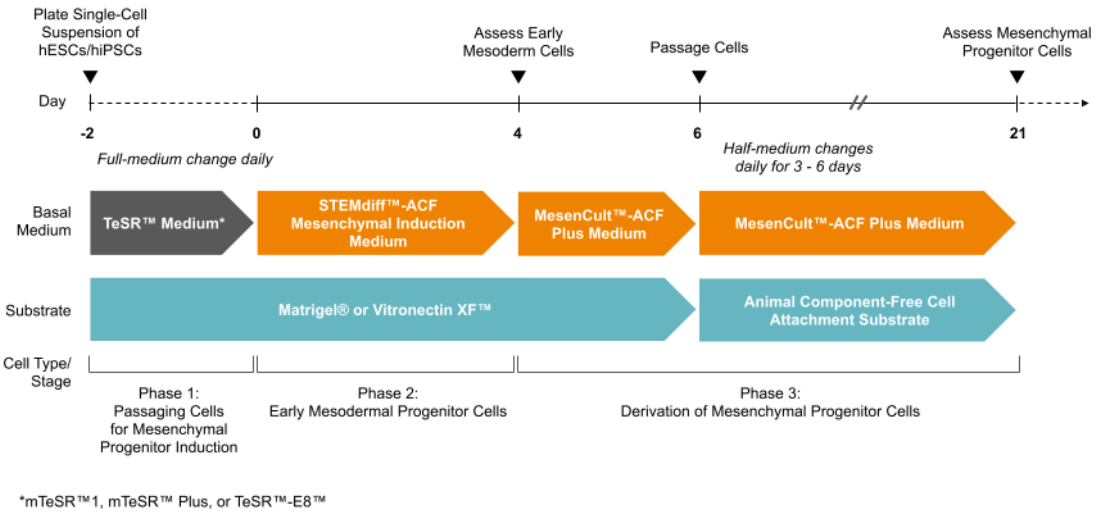
Use sterile technique to prepare complete MesenCult™-ACF Plus Medium (MesenCult™-ACF Plus Medium + MesenCult™-ACF Plus 500X Supplement + L-Glutamine). The following example is for preparing 500 mL of complete medium. If preparing other volumes, adjust accordingly.

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 aliquoted supplement, 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 thoroughly.
3. Add L-Glutamine to reach 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 freeze complete MesenCult™-ACF Plus Medium.** Do not exceed the shelf life of the individual components.

Protocol Diagram



*These cells can be expanded long-term or differentiated into adipogenic, osteogenic, or chondrogenic lineages. See Notes and Tips for details.

Directions for Use

Please read the entire protocol before proceeding. Use sterile technique when performing the following protocols.

A. Phase 1: Passaging Cells for Mesenchymal Progenitor Induction

B. Phase 2: Induction of Early Mesodermal Progenitor Cells

C. Phase 3: Derivation of Mesenchymal Progenitor Cells

The following instructions are for one well of a 6-well plate. If using other cultureware, adjust volumes accordingly.

NOTE: Only use tissue culture-treated cultureware.

A. Phase 1: Passaging Cells for Mesenchymal Progenitor Induction

This protocol is for passaging hESCs or hiPSCs cultured in mTeSR™1, mTeSR™ Plus, or TeSR™-E8™; use the medium in which the cells are routinely maintained. Throughout the protocol this will be referred to as "TeSR™ medium".

NOTE: For complete instructions on maintaining high-quality hESCs and hiPSCs for use in differentiation and coating plates with Corning® Matrigel® or Vitronectin XF™, refer to the Technical Manual for mTeSR™1, mTeSR™ Plus, or TeSR™-E8™, available at www.stemcell.com, or contact us to request a copy.

NOTE: hESCs and hiPSCs are ready for passage when the majority of colonies are large, compact, and have centers that are dense compared to their edges.

1. Coat a Falcon® 6-Well Flat-Bottom Tissue Culture-Treated Plate with Corning® Matrigel® or Vitronectin XF™ and ensure it is at room temperature (15 - 25°C) for at least 30 minutes prior to use.
2. On **day -2**, warm sufficient volumes of TeSR™ medium, DMEM/F-12, and Gentle Cell Dissociation Reagent to room temperature for passaging.
3. Prepare Single-Cell Plating Medium by adding Y-27632 (Dihydrochloride) to the medium used for cell maintenance (e.g. mTeSR™ Plus) to reach a final concentration of 10 µM.
4. Wash the well to be passaged with 1 mL of D-PBS (Without Ca++ and Mg++). Discard the wash.
5. Add 1 mL of Gentle Cell Dissociation Reagent.
6. Incubate at 37°C for 8 - 10 minutes.
7. Harvest cells by gently pipetting up and down with a P1000 micropipette set to 1 mL to achieve a single-cell suspension.
8. Transfer single-cell suspension to a 15 mL conical tube containing an equal volume of medium (DMEM/F-12 or TeSR™ medium). Rinse the well with an additional 1 - 2 mL of medium and add the rinse to the tube containing the cells.
9. Centrifuge cells at 300 x g for 5 minutes.
10. Resuspend cells in 1 mL of Single-Cell Plating Medium (prepared in step 3) and perform a viable cell count using Trypan Blue and a hemocytometer.
11. Add cells to coated plates (Corning® Matrigel® for mTeSR™1 or mTeSR™ Plus, or Vitronectin XF™ for TeSR™-E8™) in 3 mL of Single-Cell Plating Medium at a density of 5 x 10^4 cells/cm^2. If needed, adjust cell density to achieve ~30 - 50% confluency on **day -1**.

    NOTE: Additional wells may be seeded to assess the purity of early mesoderm cells by qPCR on **day 4** and assess mesenchymal progenitor cells by flow cytomety on **day 21**.
12. Incubate at 37°C for 24 hours.
13. On **day -1**, warm TeSR™ medium to room temperature.
14. Aspirate medium from the well and replace with 2 mL of fresh TeSR™ medium. Incubate at 37°C for 24 hours.
15. Continue to section B.

B. Phase 2: Induction of Early Mesodermal Progenitor Cells

1. On **day 0**, warm thawed STEMdiff™-ACF Mesenchymal Induction Medium (see Preparation of Media) to room temperature (15 - 25°C).
2. Aspirate medium from the well. Gently wash with 1 mL of D-PBS. Discard the wash.
3. Add 3 mL of STEMdiff™-ACF Mesenchymal Induction Medium per well.
4. Incubate at 37°C for 24 hours.
5. On **days 1 - 3**, perform a daily medium change with 3 mL of STEMdiff™-ACF Mesenchymal Induction Medium. Incubate at 37°C.
6. On **day 4**, cells are ready for derivation of mesenchymal progenitor cells. Continue to section C.

C. Phase 3: Derivation of Mesenchymal Progenitor Cells

1. On **day 4**, warm complete MesenCult™-ACF Plus Medium (see Preparation of Media) to room temperature (15 - 25°C).

    NOTE: Do not incubate at 37°C.
2. Aspirate medium from the well. Gently wash with 1 mL of D-PBS. Discard the wash.

3. Add 2 mL of complete MesenCult™-ACF Plus Medium.
4. Incubate at 37°C for 24 hours.
5. On **day 5**, perform a medium change with 2 mL of complete MesenCult™-ACF Plus Medium. Incubate at 37°C for 24 hours.
6. On **day 6**, passage cells onto a Costar® 6-Well Flat-Bottom Tissue Culture-Treated Plate coated with Animal Component-Free Cell Attachment Substrate, as described below.

   NOTE: For instructions on coating cultureware with Animal Component-Free Cell Attachment Substrate, refer to the Product Information Sheet for MesenCult™-ACF Plus Medium (Document #10000003462), available at www.stemcell.com, or contact us to request a copy.

   a. Warm complete MesenCult™-ACF Plus Medium to room temperature. Add Y-27632 (Dihydrochloride) to reach a final concentration of 10 µM.

      NOTE: Do not incubate at 37°C.

   b. Wash the well to be passaged once with 2.5 mL of D-PBS. Discard the wash.
   c. Add 1 mL of Gentle Cell Dissociation Reagent and incubate at 37°C for 8 - 10 minutes.
   d. Harvest cells by gently pipetting up and down with a P1000 micropipette set to 1 mL to detach cells. Transfer to a polypropylene conical tube containing an equal volume of complete MesenCult™-ACF Plus Medium.
   e. Wash the well with an additional 1 - 2 mL of complete MesenCult™-ACF Plus Medium and check wells under the microscope. If there are still some adherent cells, use a cell scraper to gently remove the remaining cells and place into the same polypropylene tube as in step d. Add ~5 mL of complete MesenCult™-ACF Plus Medium to the tube.
   f. Centrifuge the tube at 300 x g for 7 minutes.
   g. Discard the supernatant and resuspend the cell pellet in ~0.5 mL of complete MesenCult™-ACF Plus Medium with 10 µM Y-27632 (Dihydrochloride).
   h. Count viable cells using Trypan Blue and a hemocytometer.
   i. Plate cells on cultureware coated with Animal Component-Free Cell Attachment Substrate and containing 3 mL of complete MesenCult™-ACF Plus Medium with 10 µM Y-27632 (Dihydrochloride) per well.

      NOTE: Refer to Table 1 for recommended plating densities.

   j. Incubate at 37°C. Perform a daily half-medium change for approximately 3 - 6 days (cell line-dependent). When cells are approximately 80% confluent, proceed to step 7 for passaging.

7. For passage 2 and higher, use the following passaging protocol:
   a. Warm ACF Enzymatic Dissociation Solution, ACF Enzyme Inhibition Solution, and complete MesenCult™-ACF Plus Medium to room temperature.

      NOTE: Do not incubate at 37°C.

   b. Wash the well once with 2.5 mL of D-PBS. Discard the wash.
   c. Add 1 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.

   d. Add 1 mL of ACF Enzyme Inhibition Solution and collect cells in a polypropylene tube.
   e. Wash the well with 2 mL of complete MesenCult™-ACF Plus Medium and check the wells under a microscope. If > 20% of the cells remain attached, use a cell scraper to gently detach cells. Transfer to the polypropylene tube from step d.
   f. Centrifuge the tube at 300 x g for 8 minutes.
   g. Discard the supernatant. Resuspend the cell pellet in ~0.5 mL complete MesenCult™-ACF Plus Medium.
   h. Count viable cells using Trypan Blue and a hemocytometer.
   i. Plate cells on a Corning® 6-Well Flat-Bottom Tissue Culture-Treated Plate coated with Animal Component-Free Cell Attachment Substrate and containing 2 mL of complete MesenCult™-ACF Plus Medium per well. Refer to **Table 1** for recommended plating densities.
   j. Incubate cells at 37°C for approximately 3 - 6 days (cell line-dependent). Passage cells when they are approximately 80% confluent.

      NOTE: Half-medium changes are only required if cells start to detach, and are normally not required after passage 2 or 3.

Table 1. Recommended Cell Plating Densities

PASSAGE #	CELL PLATING DENSITY (cells/cm^2)	EXAMPLE OF NUMBER OF CELLS PER WELL OF A 6-WELL PLATE
0 - 1	1.5 - 10 x 10^3	1.5 - 10 x 10^4
1 - 2	3 - 8 x 10^3	3 - 8 x 10^4
2 - 3	1.5 - 6 x 10^3	1.5 - 6 x 10^4
3 - 4+	1.5 - 3 x 10^3	1.5 - 3 x 10^4

Assessment of Mesenchymal Progenitor Cells

Purity of early mesoderm cells can be measured by qPCR (increased expression of Brachyury, NCAM, and MIX-L1 with reduced expression of OCT4, SOX2, Nanog, and EpCAM) on **day 4**.

Assessment of mesenchymal progenitor cells can be verified on **day 21** by flow cytometry after labeling with fluorochrome-conjugated antibodies (see list below for examples). On **day 21**, > 90% of cells 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. Results may vary depending on cell line used.

- MSC Characterization Antibody Panel (Catalog #100-0354)
- Anti-Human CD73 (Ecto-5'-Nucleotidase) Antibody, Clone AD2 (Catalog #60044)
- Anti-human CD105 antibody, clone SN6
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018)
- Anti-Human CD34 Antibody, Clone 581 (Catalog #60013), or Clone 8G12 (Catalog #60121)
- Anti-Human OCT4 (OCT3) Antibody, Clone 40 (Catalog #60059), or Clone 3A2A20 (Catalog #60093)
- Anti-Human TRA-1-60 Antibody, Clone TRA-1-60R (Catalog #60064)

Notes and Tips

- By **day 21**, the cells should be MPC-like in terms of cell morphology and phenotype, and should possess trilineage differentiation potential (osteogenic, chondrogenic, and adipogenic). MesenCult™ media are available for adipogenic differentiation (Catalog #05412), osteogenic differentiation (Catalog #05465), and chondrogenic differentiation (Catalog #05455).
- The use of polypropylene tubes (e.g. Catalog #38009 and #38010) during subculture will help to prevent the MSCs from sticking to the tubes.

Related Products

For related products, including specialized media, matrices, antibodies, cytokines, and small molecules, visit www.stemcell.com/MESworkflow, or contact us at techsupport@stemcell.com.

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