# 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 substrate. It is optimized for the derivation of cells with mesenchymal progenitor cell (MPC)-like properties from human embryonic stem (ES) cells or induced pluripotent stem (iPS) cells. This kit provides a complete workflow of defined reagents for derivation and expansion of human ES- or iPS-derived MPCs.

## 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 for 12 months from date of manufacture (MFG) on label. |
| MesenCult™-ACF Basal Medium | 05451 | 400 mL | Store at 2 - 8°C. | Stable for 10 months from date of manufacture (MFG) on label. |
| MesenCult™-ACF 5X Supplement | 05452 | 100 mL | Store at -20°C. | Stable for 24 months from date of manufacture (MFG) on label. |
| MesenCult™-ACF Attachment Substrate | 05444 | 1 mL | Store at 2 - 8°C. | Stable until expiry (EXP) date on label. |

## Materials Required But Not Included

| PRODUCT NAME | CATALOG # |
|---|---|
| Animal Component-Free Cell Dissociation Kit<br>• ACF Enzymatic Dissociation Solution<br>• ACF Enzyme Inhibition Solution | 05426 |
| Falcon® 6-Well Flat-Bottom Plate, Tissue Culture-Treated | 38016 |
| Corning® Matrigel® hESC-Qualified Matrix<br>OR<br>Vitronectin XF™ | Corning 354277<br>OR<br>07180 |
| mTeSR™1<br>OR<br>TeSR™-E8™ | 85850<br>OR<br>05940 |
| Gentle Cell Dissociation Reagent | 07174 |
| D-PBS (Without Ca++ and Mg++) | 37350 |
| DMEM/F-12 with 15 mM HEPES | 36254 |
| Y-27632 | 72302 |
| Trypan Blue | 07050 |
| L-Glutamine | 07100 |

# Preparation of Media

**Complete MesenCult™-ACF Medium**

Use sterile techniques to prepare complete MesenCult™-ACF Medium (Basal Medium + 5X Supplement + L-Glutamine). The following example is for preparing 500 mL of complete medium. If preparing other volumes, adjust accordingly.

For aliquoting either the 5X Supplement or the complete medium, polypropylene tubes are strongly recommended (e.g. Catalog #38009, 15 mL or Catalog #38010, 50 mL); see Notes and Tips.

1. Thaw MesenCult™-ACF 5X Supplement at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.

   NOTE: Precipitate may be observed in thawed supplement or in complete medium in culture over time. This will not affect performance. If precipitate is observed in thawed supplement, leave at room temperature (15 - 25°C) for 30 minutes or incubate at 37°C for 15 minutes. Mix gently by inverting the supplement.

   NOTE: Once thawed, use immediately or aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing the aliquoted supplement, use immediately. Do not re-freeze.
2. Add 100 mL of MesenCult™-ACF 5X Supplement to 400 mL of MesenCult™-ACF Basal Medium. Mix thoroughly.
3. Add 5 mL of 200 mM L-Glutamine to reach a final concentration of 2 mM. Mix thoroughly.

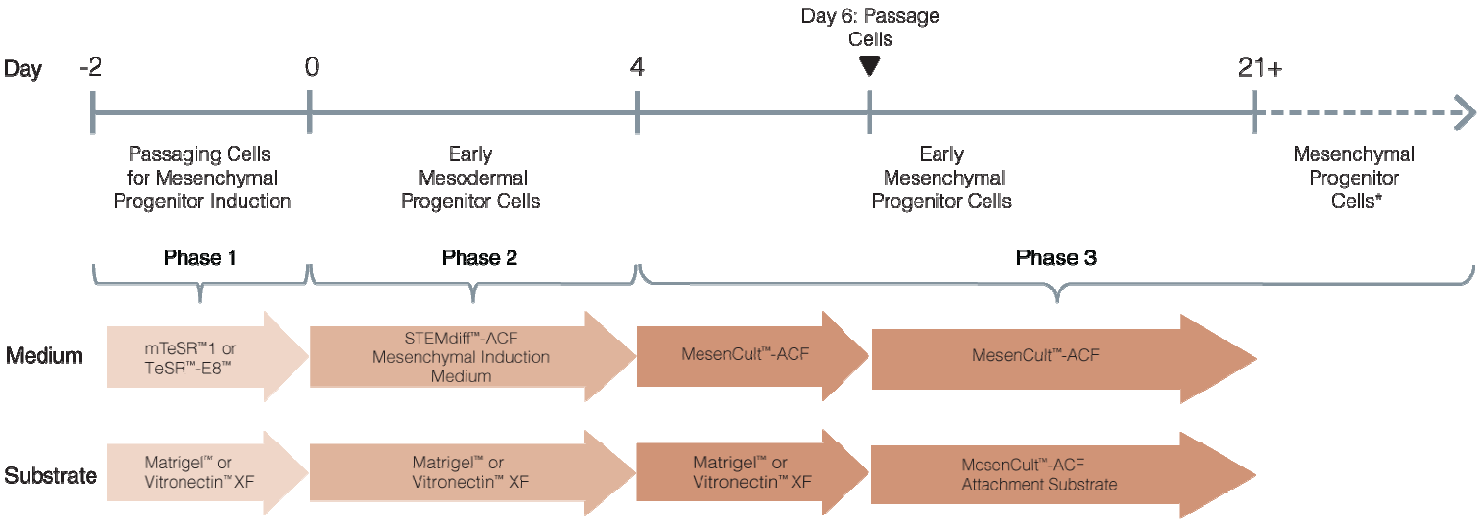
NOTE: If not used immediately, store complete MesenCult™-ACF Medium at 2 - 8°C for up to 2 weeks. Do not exceed the shelf life of the individual components.

**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.

# 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 techniques 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 1 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 human ES or iPS cells cultured in either mTeSR™1 or TeSR™-E8™; use the medium in which the cells are routinely maintained.

Coat cultureware with Corning® Matrigel® or Vitronectin XF™ and ensure it is at room temperature (15 - 25°C) for at least 30 minutes prior to use.

NOTE: For complete instructions on maintaining high-quality human ES and iPS cells for use in differentiation and coating plates with Corning® Matrigel® or Vitronectin XF™, refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™1 (Document #28315) or TeSR™-E8™ (Document #29267), available at www.stemcell.com or contact us to request a copy.

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

1. On **day -2**, warm (15 - 25°C) sufficient volumes of mTeSR™1 or TeSR™-E8™, DMEM/F-12, and Gentle Cell Dissociation Reagent for passaging.
2. Prepare Single-Cell Plating Medium by adding Y-27632 to the medium used for cell maintenance (e.g. mTeSR™1 or TeSR™-E8™) to reach a final concentration of 10 µM.
3. Wash the well to be passaged with 1 mL of D-PBS (Without Ca++ and Mg++). Discard the wash.
4. Add 1 mL of Gentle Cell Dissociation Reagent.
5. Incubate at 37°C for 8 - 10 minutes.
6. Harvest cells by gently pipetting up and down with either a serological pipette (e.g. Catalog #38001) or a 1 mL micropipette to achieve a single-cell suspension.
7. Transfer single-cell suspension to a 15 mL conical tube (e.g. Catalog #38009) containing an equal volume of medium (DMEM/F-12 or mTeSR™1 or TeSR™-E8™). Rinse the well with an additional 1 - 2 mL of medium and add the rinse to the tube containing the cells.
8. Centrifuge cells at 300 x *g* for 5 minutes.
9. Resuspend cells in 1 mL of Single-Cell Plating Medium and perform a viable cell count using Trypan Blue and a hemocytometer.
10. Plate cells onto pre-coated plates (Corning® Matrigel® for mTeSR™1 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.
11. Incubate at 37°C for 24 hours.
12. On **day -1**, warm (15 - 25°C) mTeSR™1 or TeSR™-E8™.
13. Aspirate medium from the well and replace with 2 mL of fresh mTeSR™1 or TeSR™-E8™. Incubate at 37°C for 24 hours.
14. Continue to section B.

### B) Phase 2: Induction of Early Mesodermal Progenitor Cells

1. On **day 0**, warm (15 - 25°C) thawed STEMdiff™-ACF Mesenchymal Induction Medium (see Preparation of Media).
2. Aspirate medium from the well. Gently wash with 1 mL of D-PBS (Without Ca++ and Mg++). 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 (15 - 25°C) complete MesenCult™-ACF Medium (see Preparation of Media).
2. Aspirate medium from the well. Gently wash with 1 mL of D-PBS (Without Ca++ and Mg++). Discard the wash.
3. Add 2 mL of complete MesenCult™-ACF Medium.
4. Incubate at 37°C for 24 hours.
5. On **day 5**, perform a medium change with 2 mL of complete MesenCult™-ACF Medium. Incubate at 37°C for 24 hours.
6. On **day 6**, passage cells onto cultureware pre-coated with MesenCult™-ACF Attachment Substrate as described below.

   NOTE: For instructions on coating cultureware with MesenCult™-ACF Attachment Substrate, refer to the Product Information Sheet for MesenCult™-ACF Culture Kit (Document #28066), available at www.stemcell.com or contact us to request a copy.

   a. Warm (15 - 25°C) complete MesenCult™-ACF Medium. Add Y-27632 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 (Without Ca++ and Mg++). 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 either a serological pipette (e.g. Catalog #38001) or a 1 mL micropipette to detach cells. Transfer to a polypropylene tube (e.g. Catalog #38009, 15 mL) containing an equal volume of complete MesenCult™-ACF Medium.

e. Wash the well with an additional 1 - 2 mL of complete MesenCult™-ACF Medium and check wells under the microscope. If there are still some adherent cells use a cell scraper to gently remove remaining cells and place into the same polypropylene tube as in step d. Add ~5 mL of complete MesenCult™-ACF 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 Medium with 10 µM Y-27632.

h. Count viable cells using Trypan Blue and a hemocytometer.

i. Plate cells on cultureware pre-coated with MesenCult™-ACF Attachment Substrate and containing 3 mL of complete MesenCult™-ACF Medium with 10 µM Y-27632 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 (15 - 25°C) ACF Enzymatic Dissociation Solution, ACF Enzyme Inhibition Solution, and complete MesenCult™-ACF Medium.

NOTE: Do not incubate at 37°C.

b. Wash the well once with 2.5 mL of D-PBS (Without Ca++ and Mg++). 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.g. Catalog #38009, 15 mL).

e. Wash the well with 2 mL of complete MesenCult™-ACF Medium and check wells under the 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 Medium.

h. Count viable cells using Trypan Blue and a hemocytometer.

i. Plate cells on cultureware pre-coated with MesenCult™-ACF Attachment Substrate and containing 2 mL of complete MesenCult™-ACF Medium per well.

NOTE: 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 |

## 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). STEMCELL MesenCult™ media are available for chondrogenic differentiation (Catalog #05455) and for adipogenic differentiation (Catalog #05412).
- The use of polypropylene tubes (e.g. Catalog #38009, 15 mL or Catalog #38010, 50 mL) during subculture will help to prevent the MSCs from sticking to the tubes.

## 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.

- 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)

## 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.