STEMdiff™ Mesenchymal Progenitor Kit

Animal Component-Free Kit for Differentiation of Human Pluripotent Stem Cells to Mesenchymal Progenitor Cells

The STEMdiff™ Mesenchymal Progenitor Kit (Catalog #05240) is optimized for the efficient and reproducible derivation of mesenchymal progenitor cells (MPCs) from human embryonic stem (ES) cells or induced pluripotent stem (iPS) cells in three weeks (Figure 1). This kit uses a simple monolayer protocol to generate MPCs under feeder-free conditions. Human ES- or iPS-derived MPCs are capable of long-term expansion (Figure 2), characterized by strong expression of cell surface markers CD73, CD90, CD105 and CD146, and lack expression of CD34, CD45 and CD144 (Figure 3). Human iPS- or ES-derived MPCs can be further differentiated into adipocytes, osteoblasts and chondrocytes (Figure 4).

Why Use STEMdiff™ Mesenchymal Progenitor Kit?

DEFINED. Serum- and animal component-free formulation

ROBUST. Efficient and reproducible generation of MPCs from multiple human ES or iPS cell lines

FAST. Rapid derivation of MPCs in three weeks

FUNCTIONAL. Generates MPCs capable of long-term expansion and differentiation to adipocytes, osteoblasts and chondrocytes

PRODUCT	CAPACITY	CATALOG #	COMPONENTS
STEMdiff™ Mesenchymal Progenitor Kit	1 kit	05240	• STEMdiff™-ACF Mesenchymal Induction Medium • MesenCult™-ACF Basal Medium • MesenCult™-ACF 5X Supplement • MesenCult™-ACF Attachment Substrate

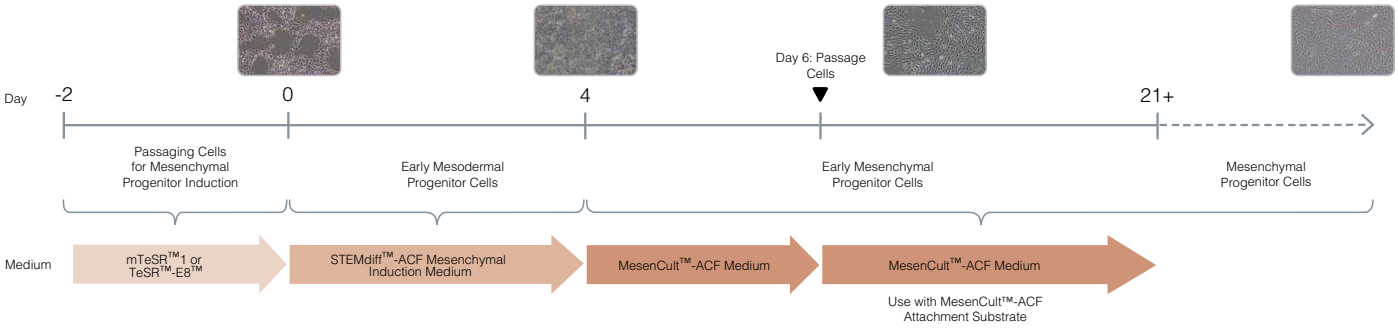

Figure 1. Schematic of Differentiation Protocol and Timeline

In Phase 1, human ES or iPS cells are cultured in mTeSR™1 or TeSR™-E8™ medium. On Day 0 (Phase 2) of the protocol, cells are ready for induction into early mesoderm progenitor cells by replacing TeSR™ medium with STEMdiff™ Mesenchymal Induction Medium. By Day 4 (Phase 3), STEMdiff™ Mesenchymal Induction Medium is replaced with MesenCult™-ACF Medium. On Day 6, cells are passaged onto cultureware precoated with MesenCult™-ACF Attachment Substrate in MesenCult™-ACF Medium to derive early MPCs. By Day 21, human ES- or iPS-derived MPCs express the suggested MPC characteristics.

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STEMdiff™
Mesenchymal Progenitor Kit

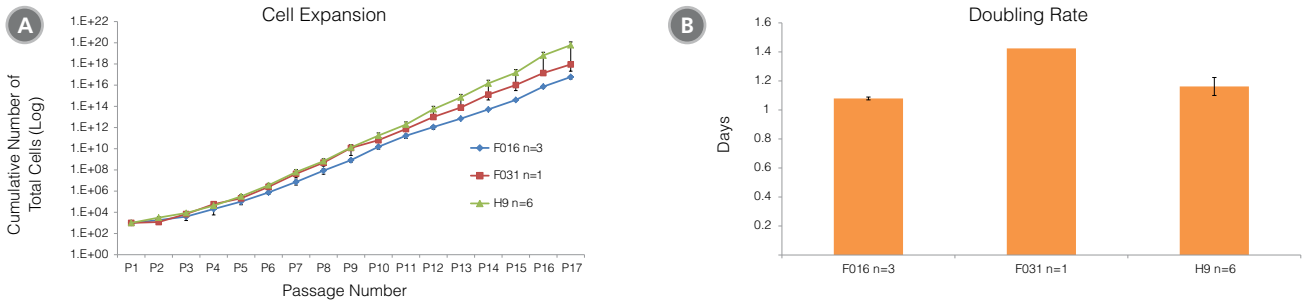

Figure 2. Cell Expansion and Doubling Rate of MPCs Derived from Human ES (H9) and iPS (STiPS-F016 and -F031) Cells in MesenCult™-ACF Medium

(A) The average cell expansion per passage over 17 passages for MPCs derived from human ES and iPS cell lines are approximately 9 and 10 fold.

(B) Days to double cell number for human ES- and iPS-derived MPCs expanded for 17 passages range from 1.1 to 1.4 days.

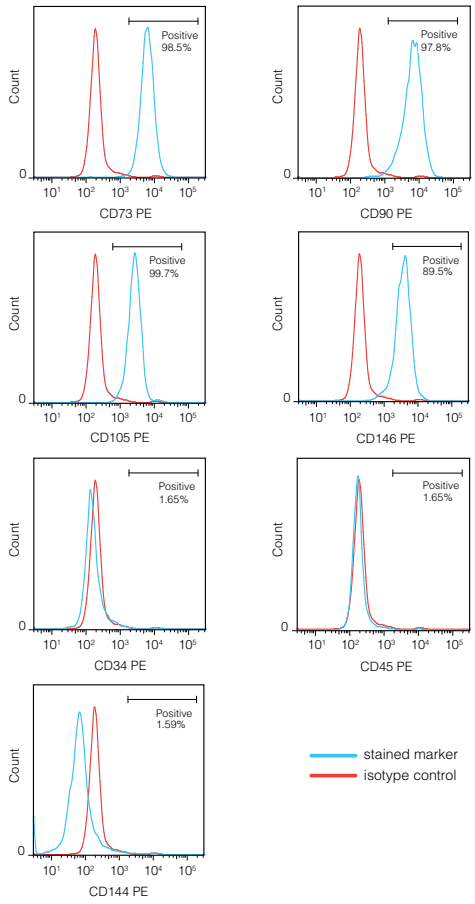

Figure 3. A Representative Flow Cytometric Analysis of Human iPS-derived MPCs Expressing Mesenchymal Surface Markers By Day 21

Human iPS-derived MPCs, generated using the STEMdiff™ Mesenchymal Progenitor Kit, express high levels of suggested mesenchymal surface markers (CD73, CD90 and CD105) and the perivascular marker, CD146. MPCs do not express hematopoietic (CD34, CD45) and endothelial (CD144) surface markers. The same phenotype is also observed in human ES-derived MPCs (data not shown).

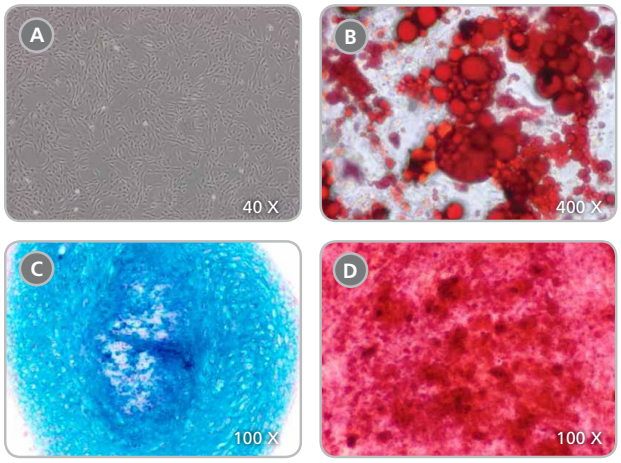

Figure 4. Human ES- and iPS-derived MPCs Can Be Further Differentiated Into Adipogenic, Chondrogenic and Osteogenic Lineages

(A) MPCs generated from the 3 week protocol (described in Figure 1) and subsequently cultured in MesenCult™-ACF Medium develop MPC-like morphology (40X magnification). These MPCs can be differentiated to (B) adipocytes (Oil Red O staining), 400X magnification; (C) chondrocytes (Alcian Blue staining), 100X magnification; and (D) osteoblasts (Fast Red and Silver Nitrate staining), 100X magnification.

The STEMdiff™ Mesenchymal Progenitor Kit is compatible with human iPS and ES cells cultured in mTeSR™1 (Catalog #05850) or TeSR™-E8™ (Catalog #05940) medium. MPCs, generated through the use of this kit, are also compatible with MesenCult™-ACF Freezing Medium (Catalog #05490) and MesenCult™-ACF Dissociation Kit (Catalog #05426). This offers a complete workflow for the derivation, expansion, cryopreservation and differentiation of MPCs from human iPS or ES cells. MPCs can be further differentiated to chondrocytes using MesenCult™-ACF Chondrogenic Differentiation Medium (Catalog #05455). Human ES- or iPS-derived MPCs can serve as a promising renewable cell source for academic and translational mesenchymal stem cell research.

For a complete list of related products, including specialized cell culture and storage media, matrices, antibodies, cytokines and small molecules, visit **www.stemcell.com/MEworkflow** or contact us at **techsupport@stemcell.com**.