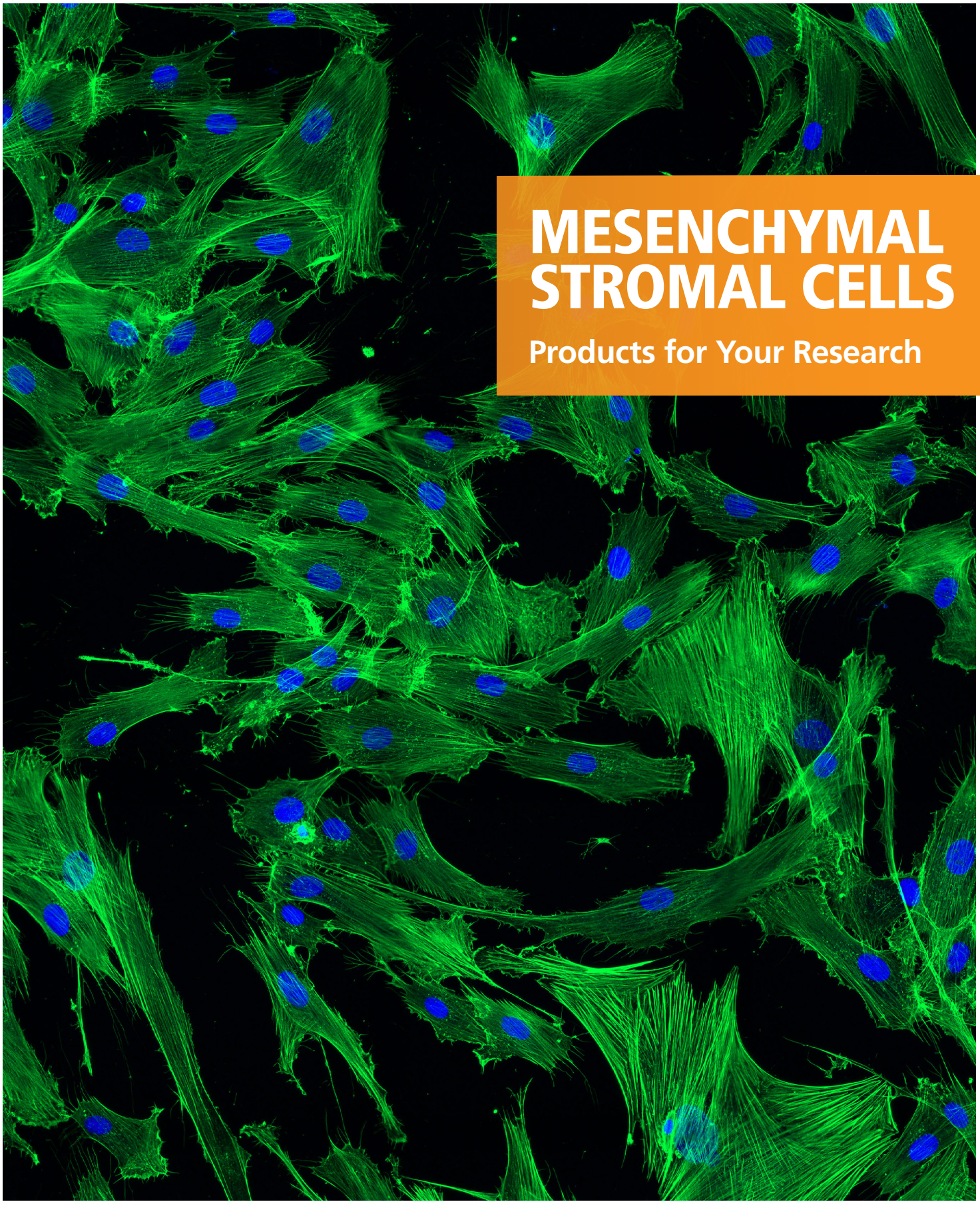

MESENCHYMAL STROMAL CELLS

Products for Your Research

STEMCELL™ TECHNOLOGIES

Scientists Helping Scientists™ | WWW.STEMCELL.COM

TABLE OF CONTENTS

Mesenchymal Stromal Cell Research

Mesenchymal stromal cells (MSCs; also known as mesenchymal stem cells, or medicinal signaling cells) are known for self-renewal and trilineage differentiation potential in vitro. MSCs can be isolated from several tissues, including bone marrow (BM), umbilical cord (UC), adipose tissue, and dental pulp. MSCs are studied to further our knowledge about their fundamental cell biology and to investigate their potential use as cell therapy agents. The MesenCult™ product line is a comprehensive and integrated suite of products for both human and mouse MSCs that enables researchers to standardize their cell culture system and minimize issues associated with performance and experimental variability.

MesenCult™-ACF Workflow

The MesenCult™-ACF workflow provides animal component-free (ACF) media and reagents to support the entire MSC culture process. This workflow enables human MSC derivation, expansion, maintenance, cryopreservation, and chondrogenic differentiation without the need for serum, other animal tissue, or body fluid. The ACF composition of MesenCult™-ACF kits enables reliable culture and characterization of MSCs. It also prevents potential contamination by animal-derived components, which in turn minimizes immunogenicity of MSCs for downstream applications. This suite of products includes the MesenCult™-ACF Plus Culture Kit (Catalog #05448), Animal Component-Free Cell Dissociation Kit (Catalog #05426), MesenCult™-ACF Freezing Medium (Catalog #05490), and MesenCult™-ACF Chondrogenic Differentiation Kit (Catalog #05455). The MesenCult™-ACF workflow also includes the STEMdiff™ Mesenchymal Progenitor Kit (Catalog #05240) for the generation of mesenchymal progenitor cells from human pluripotent stem cells (hPSCs), embryonic stem (ES) and induced pluripotent stem (iPS) cells.

STEMCELL Products for Every Step of Your MSC Research

Organism	MSC Sourcing & Isolation	Derivation, Culture & Cryopreservation	MSC Differentiation
Human	• STEMdiff™ Mesenchymal Progenitor Kit* • RosetteSep™ Human Mesenchymal Stem Cell Enrichment Cocktail • EasySep™ Human CD271 Positive Selection Kit II • iCell® Mesenchymal Stem Cells • Stromal Cells/Stromal Cells Derived in ACF Medium** • Whole Bone Marrow**	• MesenCult™-ACF Plus Medium/Culture Kit* • Animal Component-Free Cell Dissociation Kit* • MesenCult™-ACF Freezing Medium* • MesenCult™-hPL Medium Kit • MesenCult™ Proliferation Kit (Human)	• MesenCult™-ACF Chondrogenic Differentiation Kit* • MesenCult™ Adipogenic Differentiation Kit (Human) • MesenCult™ Osteogenic Differentiation Kit (Human)
Mouse	• EasySep™ Mouse Mesenchymal Stem/ Progenitor Cell Enrichment Kit	• MesenCult™ Expansion Kit (Mouse)	• MesenCult™ Adipogenic Differentiation Kit (Mouse) • MesenCult™ Osteogenic Stimulatory Kit (Mouse)

*ACF Workflow products

**Availability is subject to regional restrictions

Mesenchymal Progenitor Cell Generation from hPSCs

STEMdiff™ Mesenchymal Progenitor Kit

The STEMdiff™ Mesenchymal Progenitor Kit (Catalog #05240) is optimized for the differentiation and culture of mesenchymal progenitor cells (MPCs) from hPSCs (ES and IPS cells; Figure 1). Unlike MSCs, MPCs are not derived from the stromal compartment of tissues and exhibit reduced heterogeneity when compared to their primary cell counterparts. MPCs generated using the STEMdiff™ Mesenchymal Progenitor Kit have a high proliferation rate (Figure 2), express cell surface markers as defined by the International Society for Cell Therapy (ISCT) (Figure 3), and maintain trilineage differentiation potential (Figure 4).

Why Use the STEMdiff™ Mesenchymal Progenitor Kit?

CONSISTENT. Serum- and animal component-free formulation improves experimental reproducibility.

ROBUST. Reproducible generation of MPCs from multiple human ES or iPS cell types.

EFFICIENT. Rapid differentiation of MPCs from hPSCs in three weeks.

FUNCTIONAL. Generation of MPCs capable of long-term expansion and trilineage differentiation.

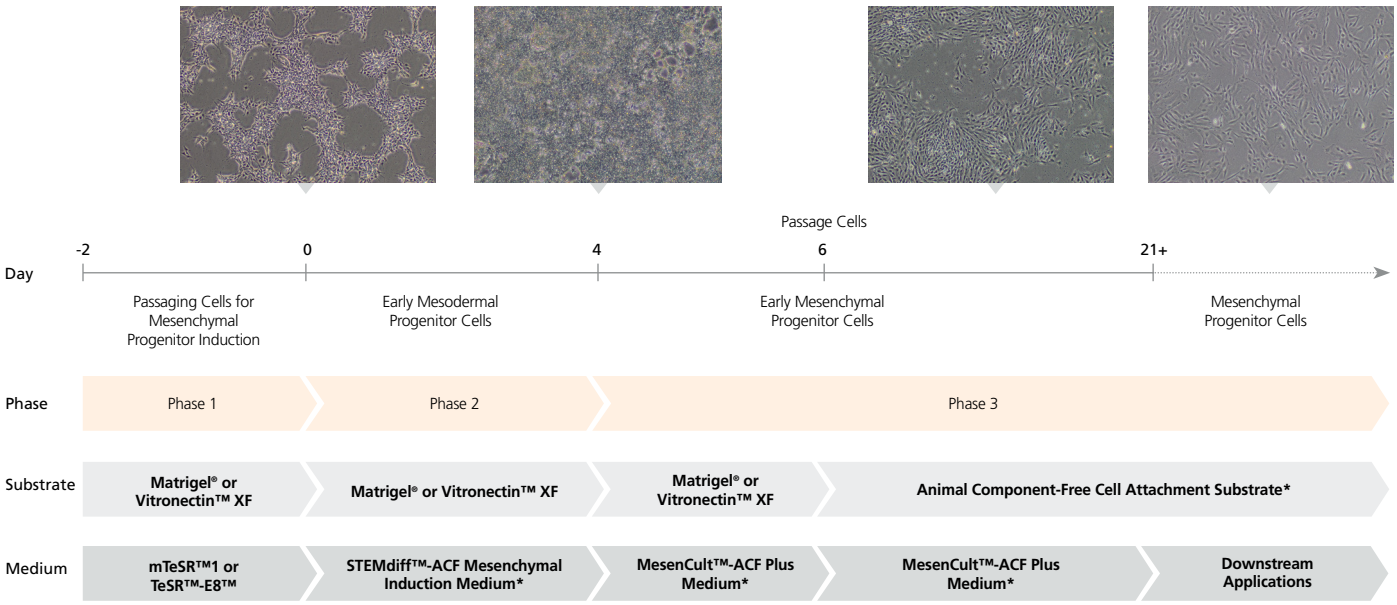

Figure 1. Schematic of hPSC-Derived MPC Differentiation Protocol

In Phase 1, hPSCs are cultured in mTeSR™1 (Catalog #85850) or TeSR™-E8™ (Catalog #05990) medium onto cultureware pre-coated with Vitronectin XF™ (Catalog #07180) or Corning® Matrigel® hESC-Qualified Matrix. On Day 0 (Phase 2) of the protocol, cells are ready for induction into early mesoderm progenitor cells by replacing TeSR™ medium with STEMdiff™-ACF Mesenchymal Induction Medium. By Day 4 (Phase 3), STEMdiff™-ACF Mesenchymal Induction Medium is replaced with complete MesenCult™-ACF Plus Medium to derive early MPCs. On Day 6, cells are passaged onto cultureware pre-coated with Animal Component-Free Cell Attachment Substrate. By Day 21, differentiated cells exhibit MPC characteristics and can be further expanded up to 20 passages.

*Components included in the STEMdiff™ Mesenchymal Progenitor Kit

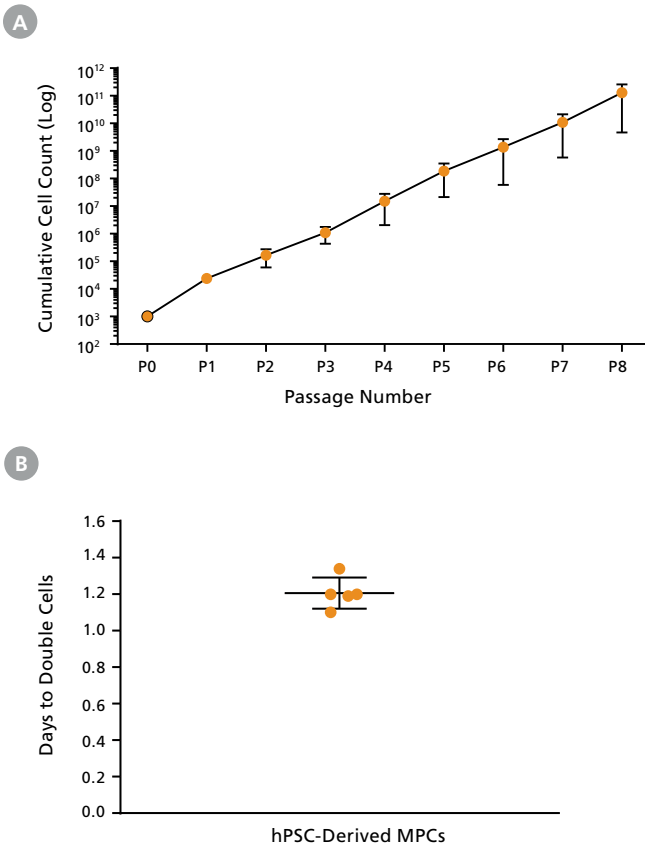

Figure 2. hPSC-Derived MPCs Generated Using the STEMdiff™ Mesenchymal Progenitor Kit Exhibit High Rate of Cell Expansion in MesenCult™-ACF Plus Medium

Human MPCs generated from hPSCs using the STEMdiff™ Mesenchymal Progenitor Kit show high (A) cumulative cell expansion and (B) average time-to-double culture of 1.2 days (P3 - P8). Data shown here represent iPS (n = 1) and ES (n = 4) cells differentiated using the STEMdiff™ Mesenchymal Progenitor Kit and expanded using the MesenCult™ ACF-Plus Culture Kit. Error bars represent standard error of mean (SEM).

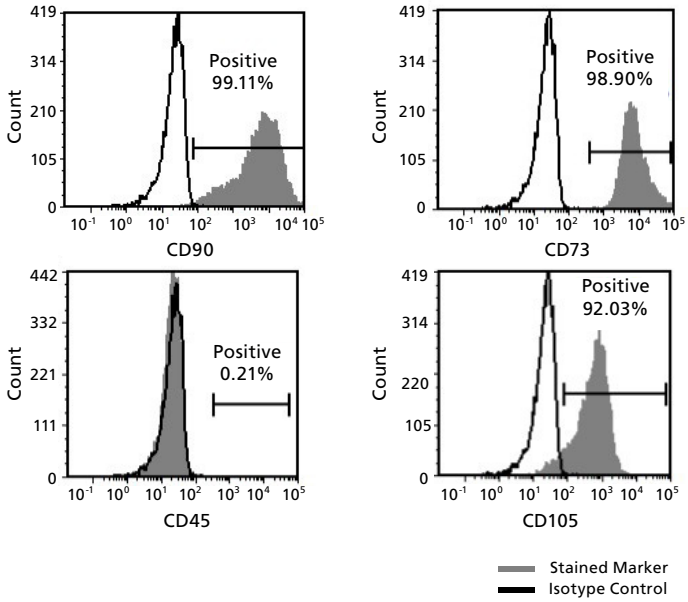

Figure 3. Flow Cytometry Analysis of iPS Cell-Derived MPCs Cultured in the STEMdiff™ Mesenchymal Progenitor Kit

MPCs were generated from human iPS cells using the STEMdiff™ Mesenchymal Progenitor Kit and expanded using MesenCult™ ACF-Plus Medium. These MPCs met MSC surface marker criteria as defined by the ISCT (CD73, CD90, and CD105), and did not express the hematopoietic marker CD45 at 23 days post-differentiation. MPCs derived from human ES cells exhibited the same phenotype (data not shown).

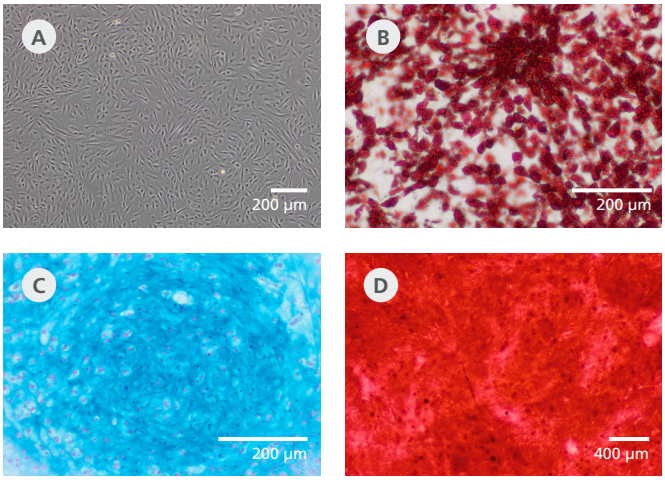

Figure 4. ES Cell-Derived MPCs Maintain Robust Differentiation Capacity

Human ES cells were (A) differentiated into MPCs using the STEMdiff™ Mesenchymal Progenitor Kit and expanded using MesenCult™-ACF Plus Medium. The resulting MPCs were then differentiated into (B) adipocytes (Oil Red O), (C) chondrocytes (Alcian Blue and nuclear fast red), and (D) osteoblasts (Alizarin Red S) using the MesenCult™ Adipogenic Differentiation Kit (Human; Catalog #05412), MesenCult™-ACF Chondrogenic Differentiation Kit , and MesenCult™ Osteogenic Differentiation Kit (Human; Catalog #05465), respectively. Similar results were observed for iPS cell-derived MPCs (data not shown).

Human MSC Derivation, Culture, and Cryopreservation

MesenCult™-ACF Plus Medium

MesenCult™-ACF Plus Medium/Culture Kit (Catalog #05445/48) is a standardized animal component-free medium kit for the derivation and culture of human MSCs from BM, UC, and adipose tissue. Compared to serum-free or xeno-free formulations, MSCs derived and expanded in MesenCult™-ACF Plus Medium generate an equal or greater total number of colony forming unit-fibroblasts (CFU-Fs; Figure 5) and a greater total number of cells per passage (Figure 6). MesenCult™ ACF-Plus Medium has superior or equal performance when compared to FBS- or human platelet lysate (hPL)-containing media, respectively (Figure 7). The MSCs derived and expanded using MesenCult™ ACF-Plus Medium retain a robust expansion rate and the potential for in vitro trilineage differentiation (Figure 8), and express cell surface markers as defined by the ISCT (Figure 9).

Why Use MesenCult™-ACF Plus Medium?

CONSISTENT. Animal component-free formulation improves experimental reproducibility.

HIGH PERFORMANCE. Superior cell expansion when compared to serum-containing media.

FUNCTIONAL. Cultured MSCs retain robust expansion and trilineage differentiation capacity.

OPTIMIZED. Supports MSC derivation directly from primary human tissue without the addition of human serum.

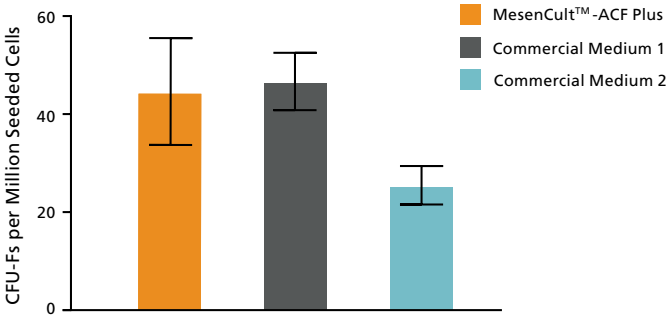

Figure 5. MesenCult™-ACF Plus Medium Derives MSCs From BM Without the Addition of Human Serum

An average of 45 CFU-Fs per million seeded cells were observed when human BM-mononuclear cells were seeded in MesenCult™-ACF Plus Medium (n = 4). An average of 47 and 25 CFU-Fs per million cells were observed for Commercial Medium 1 (n = 3) and Medium 2 (n = 4), respectively. Xeno-free and serum-free Commercial Medium 1 and Commercial Medium 2 were supplemented with 2.5% human AB serum as per their protocols for MSC derivation. No addition of serum is required when using MesenCult™-ACF Plus Medium. Error bars indicate SEM.

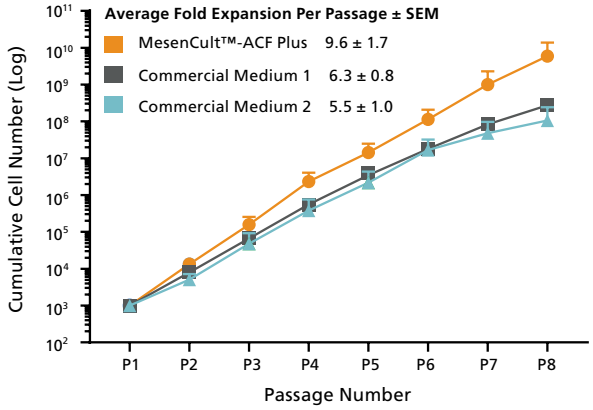

Figure 6. BM-Derived MSCs Expand Rapidly in MesenCult™-ACF Plus Medium

A greater number of human BM-derived MSCs were generated using MesenCult™-ACF Plus Medium (n = 4) when compared to xeno-free and serum-free Commercial Medium 1 (n = 3) and Commercial Medium 2 (n = 2). Over 8 passages, MSCs cultured in MesenCult™-ACF Plus Medium underwent an average of 9.6-fold expansion per passage compared to 6.3- and 5.5-fold for Commercial Medium 1 and Commercial Medium 2, respectively. Error bars represent SEM.

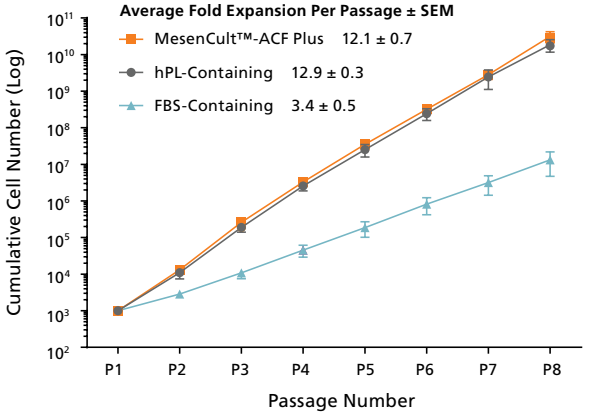

Figure 7. MesenCult™-ACF Plus Medium Has Superior or Equal Performance Compared to FBS- or hPL-Containing Media

Human BM-derived MSCs expand equally or more efficiently in MesenCult™ ACF Medium compared to hPL- or FBS-containing media, MesenCult™-hPL Medium Kit (Catalog #05439) and MesenCult™ Proliferation Kit (Human; Catalog #05411). Error bars represent SEM (n = 5).

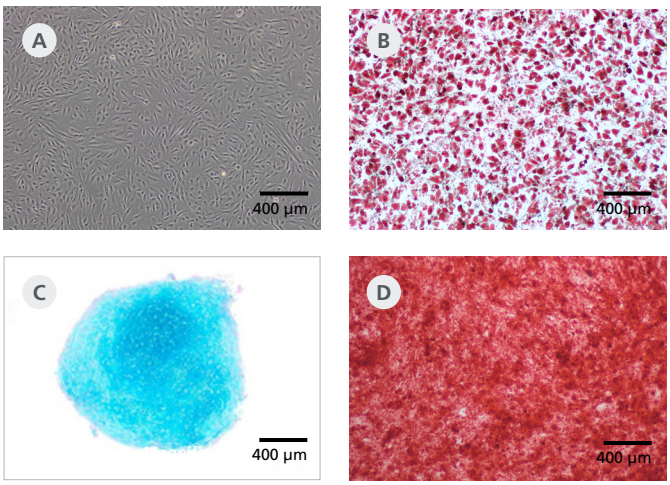

Figure 8. BM-Derived MSCs Expanded in MesenCult™-ACF Plus Medium Display Trilineage Differentiation Potential In Vitro

Human BM-derived MSCs (A) expanded in MesenCult™-ACF Plus Medium (P2) were differentiated into (B) adipocytes (Oil Red O), (C) chondrocytes (Alcian Blue and nuclear fast red), and (D) osteoblasts (Alizarin Red S) using the MesenCult™ Adipogenic Differentiation Kit (Human), MesenCult™-ACF Chondrogenic Differentiation Kit, and MesenCult™ Osteogenic Differentiation Kit (Human), respectively.

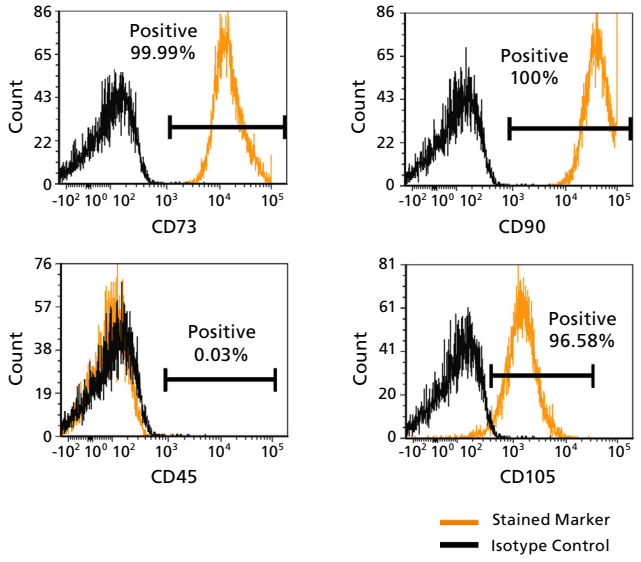

Figure 9. Flow Cytometry Analysis of BM-Derived MSCs Derived and Expanded in MesenCult™-ACF Plus Medium

Human BM-derived MSCs were derived and expanded in MesenCult™-ACF Plus Medium. MSCs from passage 8 expressed high levels of CD73, CD90, and CD105, but did not express the hematopoietic marker CD45, fulfilling the MSC surface marker criteria defined by the ISCT.

Animal Component-Free Cell Dissociation Kit

The animal Component-Free Cell Dissociation Kit (Catalog #05426) is optimized for dissociation and passaging of human stem and progenitor cells, including MSCs cultured in various media formulations. This kit includes the ACF Enzymatic Dissociation Solution and the ACF Enzyme Inhibition Solution, and is part of the MesenCult™-ACF workflow.

MesenCult™-ACF Freezing Medium

MesenCult™-ACF Freezing Medium (Catalog #05490) is an animal component-free medium for the cryopreservation of MSCs and hPSC-derived MPCs and is part of the MesenCult™-ACF workflow. This complete and ready-to-use medium is recommended for the cryopreservation of MSCs previously cultured in MesenCult™-ACF Plus Medium, the MesenCult™ Proliferation Kit (Human), or the MesenCult™-hPL Medium Kit. MSCs and MPCs cryopreserved in MesenCult™-ACF Freezing Medium have high recovery and viability (Figure 10) and maintain robust expansion and trilineage capacities post-thaw (data not shown). Frozen human MSCs should be stored at -135°C (liquid nitrogen) or colder.

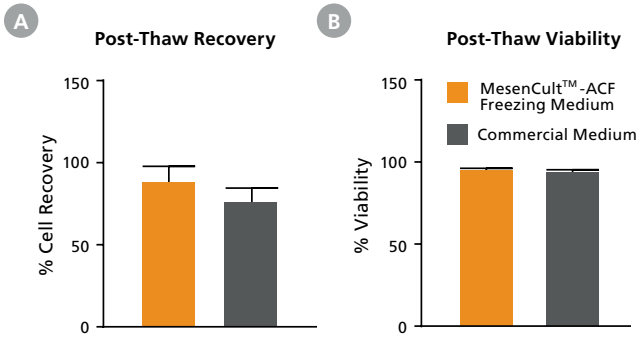

Figure 10. Cell Recovery and Viability in MesenCult™-ACF Freezing Medium

MSCs cryopreserved using MesenCult™-ACF Freezing Medium have (A) higher post-thaw recovery (number of cells recovered / number of cells frozen) than competitor ACF freezing medium and (B) maintain high viability (number of live cells / total number of cells). Error bars represent SEM (n = 5).

MesenCult™-hPL Medium Kit

MesenCult™-hPL Medium Kit (Catalog #05439) is optimized for the derivation and expansion of MSCs from human BM. This medium contains a proprietary fibrinogen-depleted human platelet lysate (hPL), an alternative growth supplement to fetal bovine serum (FBS). Complete MesenCult™-hPL Medium Kit does not require addition of growth factors, lipids, anti-coagulants such as heparin, or attachment substrate. This medium kit is more consistent and provides better expansion performance than FBS-containing media (Figure 11). MSCs culture-expanded using the MesenCult™-hPL Medium Kit express high levels of MSC surface markers as defined by the ISCT (Figure 12), and maintain trilineage differentiation potential (Figure 13).

Why Use MesenCult™-hPL Medium Kit?

CONSISTENT. FBS-free formulation improves experimental reproducibility.

FUNCTIONAL. Cultured MSCs retain robust expansion and differentiation capacities.

HIGH PERFORMANCE. Superior cell expansion compared to FBS-containing media.

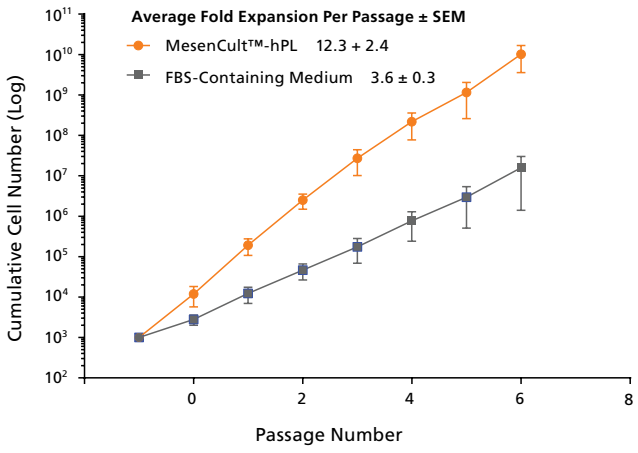

Figure 11. BM-Derived MSCs Cultured in the MesenCult™-hPL Medium Kit Expand Faster Than Those Cultured in FBS-Containing Medium

Human mononuclear BM cells were directly cultured and expanded in the MesenCult™-hPL Medium Kit or the FBS-containing MesenCult™ Proliferation Kit. Cell expansion was measured over a period of 8 passages, (n = 3). Error bars represent SEM.

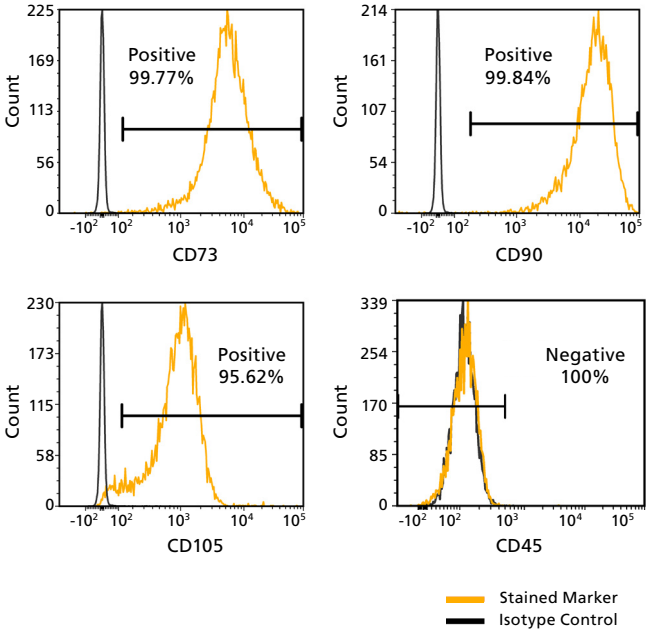

Figure 12. Flow Cytometry Analysis of MSCs Expanded in the MesenCult™-hPL Medium Kit

Human BM-derived MSCs were derived and culture-expanded in the MesenCult™-hPL Medium Kit and analyzed by flow cytometry. These cells expressed high levels of MSC surface markers as defined by the ISCT (CD73, CD90, and CD105) and lacked expression of the hematopoietic marker CD45.

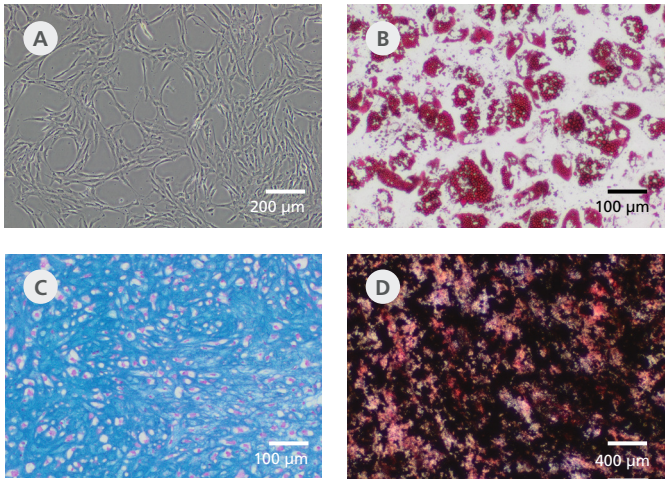

Figure 13. MSCs Derived and Cultured in the MesenCult™-hPL Medium Kit Display Trilineage Differentiation Potential

(A) Human BM-derived MSCs derived and cultured in the MesenCult™-hPL Medium Kit were differentiated into (B) adipocytes (Oil Red O), (C) chondrocytes (Alcian Blue and nuclear fast red), and (D) osteoblasts (alkaline phosphatase and von Kossa) using the MesenCult™ Adipogenic Differentiation Kit (Human), MesenCult™-ACF Chondrogenic Differentiation Kit, and MesenCult™ Osteogenic Differentiation Kit (Human), respectively.

MesenCult™ Proliferation Kit (Human)

MesenCult™ Proliferation Kit (Human; Catalog #05411) is a standardized serum-containing kit for the culture of human MSCs. This kit is optimized for the derivation and expansion of human MSCs in vitro as well as detection and enumeration of CFU-Fs. Using this kit, MSCs can be derived from BM (Figure 14), UC Wharton's jelly (Figure 15), and adipose tissue (data not shown). MSCs derived and expanded using this kit rapidly proliferate (Figure 16), while expressing cell surface markers as defined by the ISCT (Figure 17). These MSCs also retain robust trilineage differentiation potential (Figure 18).

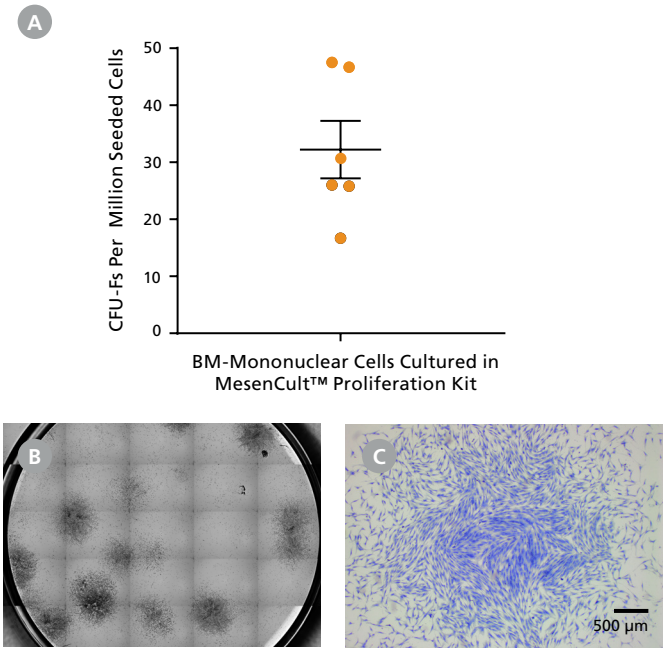

Figure 14. MesenCult™ Proliferation Kit (Human) Enriches CFU-Fs from BM

(A) An average of 32 CFU-Fs per million mononuclear cells seeded was observed when human BM was cultured in the MesenCult™ Proliferation Kit (Human). (B) Numerous CFU-Fs were observed in BM mononuclear cultures maintained in the MesenCult™ Proliferation Kit (Human) for 12 days and at seeding density of 3.0 x 10^4 cells/cm^2. (C) Representative image of a single CFU-F colony expanded for 12 days in the MesenCult™ Proliferation Kit and stained with toluidine blue. Error bar represents SEM.

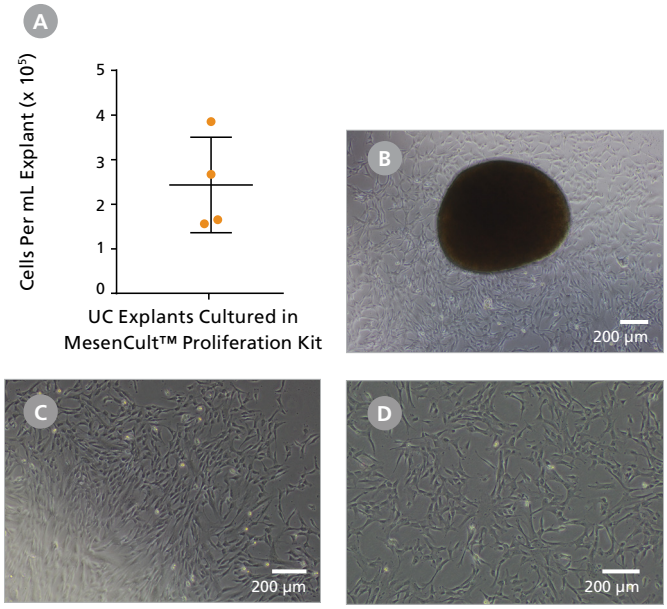

Figure 15. MSCs Derived from UC Explants Rapidly Expand When Cultured in MesenCult™ Proliferation Kit (Human)

(A) An average of 2.5x10^5 cells per mL of explants was achieved when UC explants were expanded in the MesenCult™ Proliferation Kit (Human) over the course of 10 days. MSCs derived from UC on (B) day 7 and (C) day 10 of initial culture, and (D) after 6 passages in the MesenCult™ Proliferation Kit (Human).

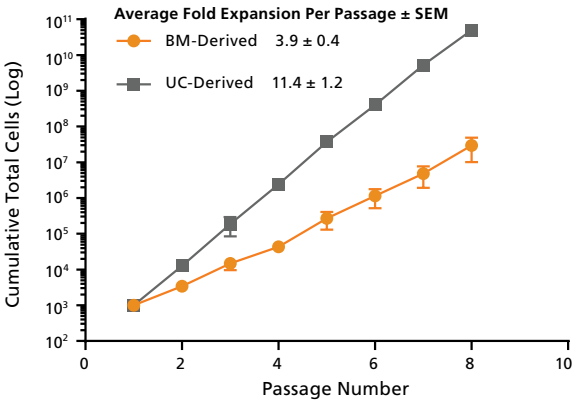

Figure 16. MesenCult™ Proliferation Kit Rapidly Expands Human UC- and BM-Derived MSCs

MSCs were derived from human BM and UC and expanded over 8 passages. An average fold expansion of 3.9 and 11.4 was observed for BM and UC, respectively (n = 5 and n = 4 for BM and UC, respectively). Error bars represent SEM.

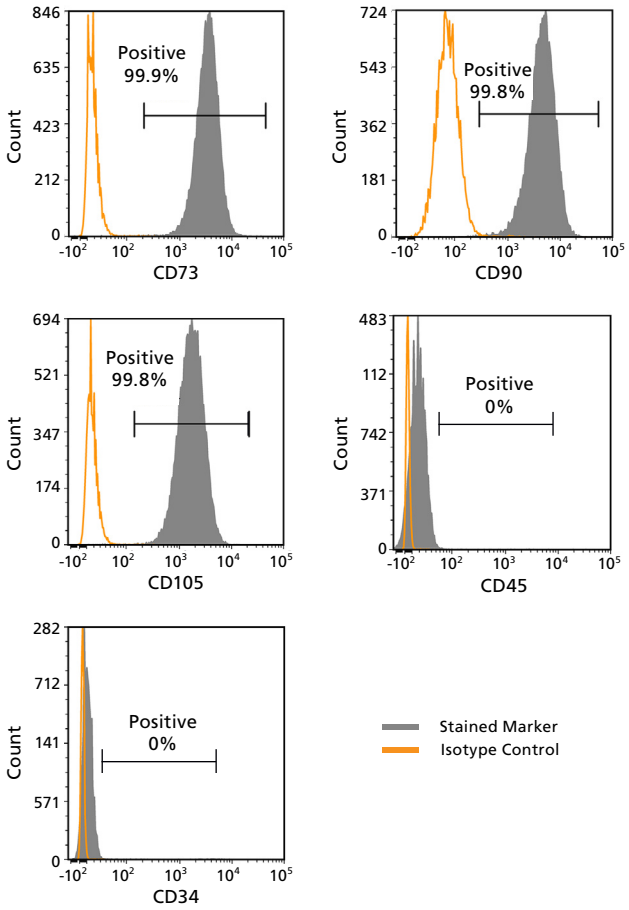

Figure 17. MSCs Expanded in the MesenCult™ Proliferation Kit (Human) Express the ISCT-Defined Surface Markers for MSCs

MSCs derived from BM and expanded using the MesenCult™ Proliferation Kit (Human) show high expression of the ISCT-defined MSC markers (CD73, CD90, and CD105) and lack expression of hematopoietic markers CD34 and CD45 (P5).

Why Use the MesenCult™ Proliferation Kit (Human)?

VERSATILE. Derives and expands MSCs from BM, UC and adipose tissue.

REPRODUCIBLE. Rigorous raw material screening and quality control ensure minimal lot-to-lot variability.

EFFICIENT. Rapid derivation and expansion of MSCs.

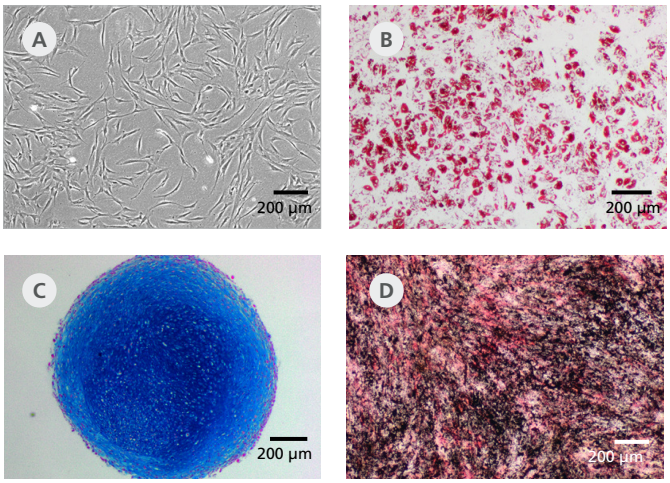

Figure 18. MSCs Expanded in the MesenCult™ Proliferation Kit (Human) Retain Trilineage Differentiation Potential

(A) Human BM-derived MSCs cultured in the MesenCult™ Proliferation Kit (Human) were differentiated into (B) adipocytes (Oil Red O), (C) chondrocytes (Alcian Blue and nuclear fast red), and (D) osteoblasts (alkaline phosphatase and von Kossa) using the MesenCult™ Adipogenic Differentiation Kit (Human), MesenCult™-ACF Chondrogenic Differentiation Kit, and MesenCult™ Osteogenic Differentiation Kit (Human), respectively.

Human MSC Differentiation

MesenCult™ Adipogenic Differentiation Kit (Human)

MesenCult™ Adipogenic Differentiation Kit (Human; Catalog #05412) is optimized for the in vitro differentiation of human MSCs into adipogenic lineage cells. This kit is suitable for differentiating human BM-, adipose tissue-, and UC-derived MSCs that have been previously cultured in media such as MesenCult™-ACF Plus Medium (Figure 8B), the MesenCult™-hPL Medium Kit (Figure 13B), and the MesenCult™ Proliferation Kit (Human) (Figure 18B); or hPSC-derived MPCs generated using the STEMdiff™ Mesenchymal Progenitor Kit (Figure 4B).

MesenCult™ Osteogenic Differentiation Kit (Human)

MesenCult™ Osteogenic Differentiation Kit (Human; Catalog #05465) is formulated for the in vitro differentiation of human MSCs into osteogenic lineage cells. This kit is suitable for the differentiation of human BM- and adipose tissue-derived MSCs that have been previously cultured in media such as MesenCult™-ACF Plus Medium (Figure 8D), the MesenCult™-hPL Medium Kit (Figure 13D), and the MesenCult™ Proliferation Kit (Human; Figure 18D); or hPSC-derived MPCs generated using the STEMdiff™ Mesenchymal Progenitor Kit (Figure 4D).

MesenCult™-ACF Chondrogenic Differentiation Kit

MesenCult™-ACF Chondrogenic Differentiation Kit (Catalog #05455) is an ACF formulation for the in vitro differentiation of human MSCs into chondrogenic lineage cells, including chondrocytes. This medium is suitable for the differentiation of MSCs derived from human BM and adipose tissue that have been previously cultured in media such as MesenCult™-ACF Plus Medium (Figure 8C), the MesenCult™-hPL Medium Kit (Figure 13C), and the MesenCult™ Proliferation Kit (Human; Figure 18C); or hPSC-derived MPCs generated using the STEMdiff™ Mesenchymal Progenitor Kit (Figure 4C).

Mouse MSC Culture

MesenCult™ Expansion Kit (Mouse)

MesenCult™ Expansion Kit (Mouse; Catalog #05513) is optimized to derive and expand mouse MSCs and embryonic fibroblasts (MEFs) (Figure 19). Complete MesenCult™ Expansion Medium is prepared by combining the basal medium and supplement, with or without MesenPure™ (Catalog #05500). Mouse MSCs cultured in MesenCult™ Expansion Medium (mouse)show greater long-term expansion compared to MSCs cultured in other commercial media (Figure 20). The addition of MesenPure™ to complete medium reduces hematopoietic cell contamination, resulting in enriched MSC cultures as early as passage 0. This kit is compatible for use with mouse BM-, adipose-, and compact bone-derived MSCs, and MEFs. MSCs derived and expanded using the MesenCult™ Expansion Kit (Mouse) maintain their trilineage differentiation potential (Figure 21) and express cell surface markers characteristic of mouse MSCs (Figure 22). A Hypoxia Incubator Chamber (Catalog #27310) and a Single Flow Meter (Catalog #27311) are available to create a hypoxic environment, which has been shown to improve mouse MSC expansion rates in comparison to cultures kept under normoxic conditions.[1-2]

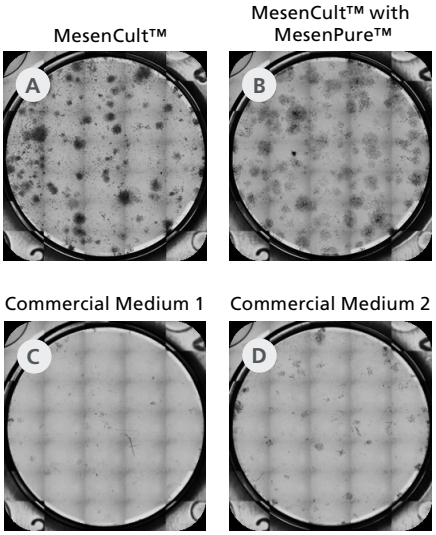

Figure 19. MesenCult™ Expansion Kit (Mouse) Can Derive CFU-Fs from Mouse BM

Numerous CFU-Fs were observed in mouse mononuclear BM cultures maintained in MesenCult™ Expansion Medium (Mouse) (A) without and (B) with MesenPure™. Few to no colonies were observed when cultures were maintained in (C) Commercial Medium 1 or (D) Commercial Medium 2 following the manufacturers' recommended protocols. Seeding density: 5.0 x 10^4 cells/cm^2. Similar results were observed for compact bone- and adipose-derived MSCs.

Why Use the MesenCult™ Expansion Kit (Mouse)?

EFFICIENT. Fast expansion of mouse MSCs with robust enrichment as early as passage 0.

VERSATILE. Optimized for use with MEFs and mouse BM-, compact bone-, and adipose tissue-derived MSC.

FUNCTIONAL. Rapid derivation and expansion of MSCs.

RELIABLE. Rigorous raw material screening and quality control minimize lot-to-lot variability and increase reproducibility between experiments.

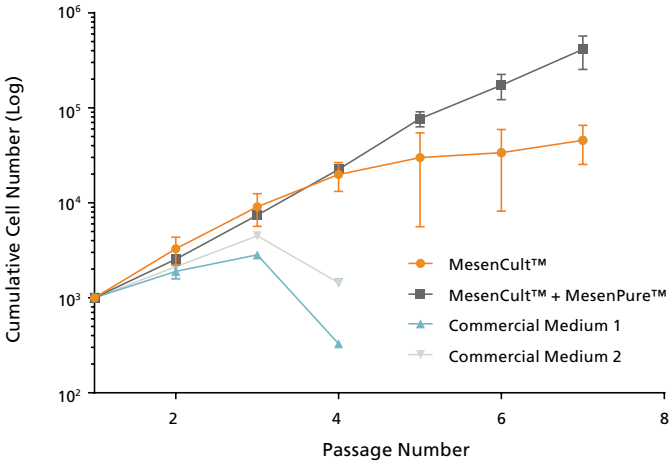

Figure 20. Mouse BM-Derived MSCs Expand Rapidly When Cultured Using the MesenCult™ Expansion Kit (Mouse)

Mouse MSCs derived and cultured in MesenCult™ Expansion Medium (Mouse) show superior long-term expansion rates compared to Commercial Medium 1 and 2. The addition of MesenPure™ improves the expansion rates at later passages and reduces the doubling time of MSCs from 3.0 ± 0.5 to 2.3 ± 0.1 days. Experiments using the MesenCult™ Expansion Kit (Mouse) were performed under hypoxic conditions. Experiments using Commercial Medium 1 and 2 were performed under normoxic conditions as recommended by manufacturers' protocols. Error bars represent SEM (n = 3 for MesenCult™ and MesenCult™ + MesenPure™).

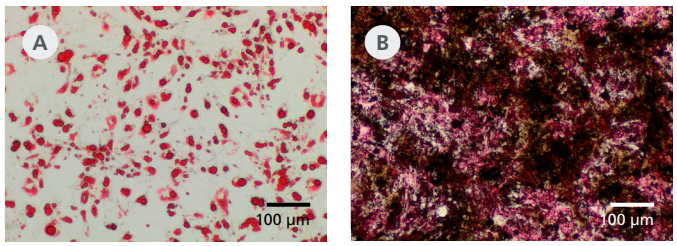

Figure 21. Mouse BM-Derived MSCs Cultured in the MesenCult™ Expansion Kit (Mouse) Maintain Multi-Lineage Differentiation Potential

Mouse BM-derived MSCs derived and cultured in MesenCult™ Expansion Medium (Mouse) with MesenPure™ were differentiated into cells of (A) adipogenic and (B) osteogenic lineages when cultured under hypoxic conditions using the MesenCult™ Adipogenic Differentiation Kit (Mouse; Catalog #05507) and MesenCult™ Osteogenic Stimulatory Kit (Mouse; Catalog #05504), respectively. Adipocytes were stained with Oil Red O for lipids, and osteoblasts were stained with silver nitrate (von Kossa) for calcification and for alkaline phosphatase activity. Compact bone- and adipose tissue-derived MSCs as well as MEFs cultured in complete MesenCult™ Expansion Medium (with or without MesenPure™) were also successfully differentiated into adipocytes and osteoblasts (data not shown).

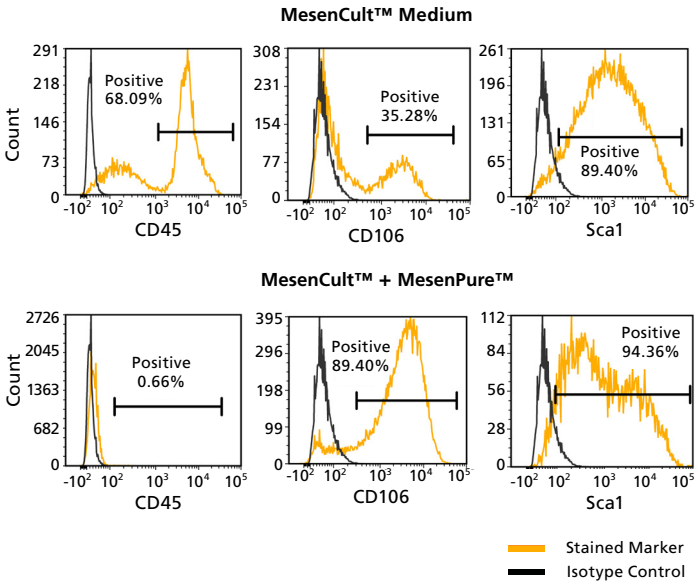

Figure 22. Mouse BM-Derived MSCs Cultured Using MesenCult™ Expansion Kit (Mouse) Exhibit Characteristic MSC Cell Surface Marker Expression

Mouse BM mononuclear cells were cultured in MesenCult™ Expansion Medium or in MesenCult™ Expansion Medium with MesenPure™. MSCs from passage 2 were immunolabeled for the mesenchymal surface markers CD106 and Sca1 as well as the hematopoietic marker CD45 and analyzed by flow cytometry. MSCs cultured in MesenCult™ Expansion Medium showed distinct populations of $CD45^+$ hematopoietic cells and $CD45^-$ /$CD106^+$/$Sca1^+$ MSCs. Upon addition of MesenPure™ to MesenCult™ Expansion Medium, an enriched and homogenous population of $CD45^-$ ($CD106^+$ and $Sca1^+$) MSCs was obtained.

MesenCult™ Adipogenic Differentiation Kit (Mouse)

MesenCult™ Adipogenic Differentiation Kit (Mouse; Catalog #05507) is specifically formulated for the in vitro differentiation of MEFs and mouse BM-, compact bone-, and adipose tissue-derived MSCs into cells of the adipogenic lineage (Figure 21A).

MesenCult™ Osteogenic Stimulatory Kit (Mouse)

MesenCult™ Osteogenic Stimulatory Kit (Mouse; Catalog #05504) is optimized for the in vitro differentiation of MEFs and mouse MSCs from compact bone, BM, and adipose tissue into cells of osteogenic lineage (Figure 21B). This kit is recommended for characterizing MSCs and MEFs and studying bone development.

Human MSC Sourcing and Isolation

RosetteSep™ Human Mesenchymal Stem Cell Enrichment Cocktail

RosetteSep™ Human Mesenchymal Stem Cell Enrichment Cocktail (Catalog #15128/68) isolates highly enriched human MSCs from human BM (Figure 23). Unwanted cells are targeted for removal with tetrameric antibody complexes recognizing CD3, CD14, CD19, CD38, CD66b, or glycophorin A on red blood cells (RBCs; Figure 24). The leukocytes and RBCs then pellet following a centrifugation step over a buoyant density medium such as Lymphoprep™ (Catalog #07801), leading to highly enriched MSCs.

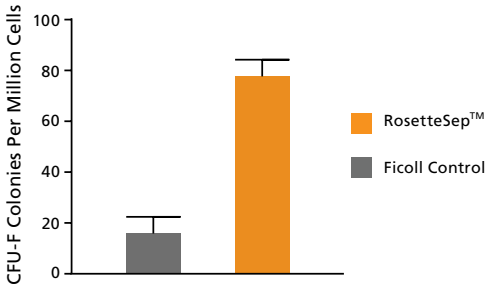

Figure 23. RosetteSep™ Human Mesenchymal Stem Cell Enrichment Cocktail Increases the Frequency of CFU-F Colonies

An average of 78 CFU-Fs per million seeded cells was observed following enrichment of human BM samples using the RosetteSep™ Human Mesenchymal Stem Cell Enrichment Cocktail (n = 3). A 4.7-fold enrichment of CFU-Fs was observed for RosetteSep™ treated samples. Error bars represent SEM.

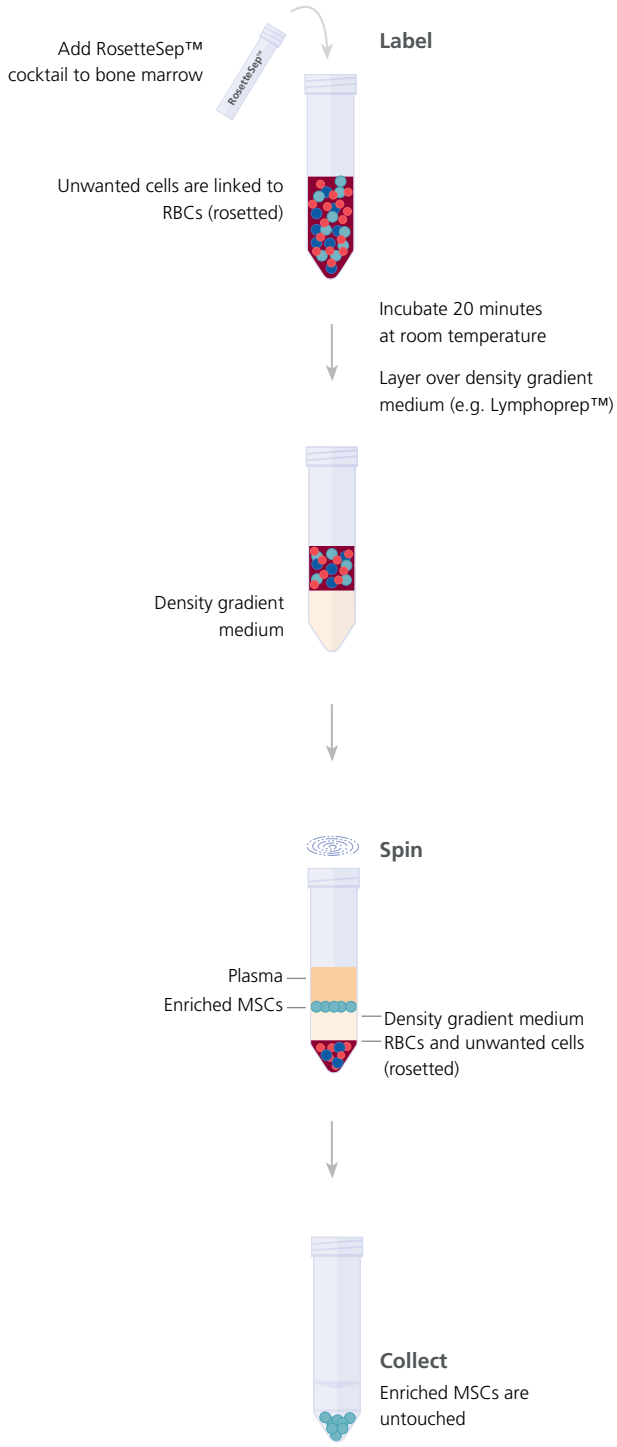

Figure 24. Overview of the RosetteSep™ Human Mesenchymal Stem Cell Enrichment Cocktail Procedure

EasySep™ Human CD271 Positive Selection Kit II

EasySep™ Human CD271 Positive Selection Kit II (Catalog #17849) isolates MSCs from human BM without the use of cell separation columns. CD271 (known as low-affinity nerve growth receptor) is recognized as a highly selective marker for the isolation and characterization of human BM-derived MSCs.[3-4] The kit positively selects MSCs using CD271-recognizing antibody complexes and magnetic particles (Figure 25). The cocktail also contains an antibody to the human Fc receptor to minimize non-specific binding.

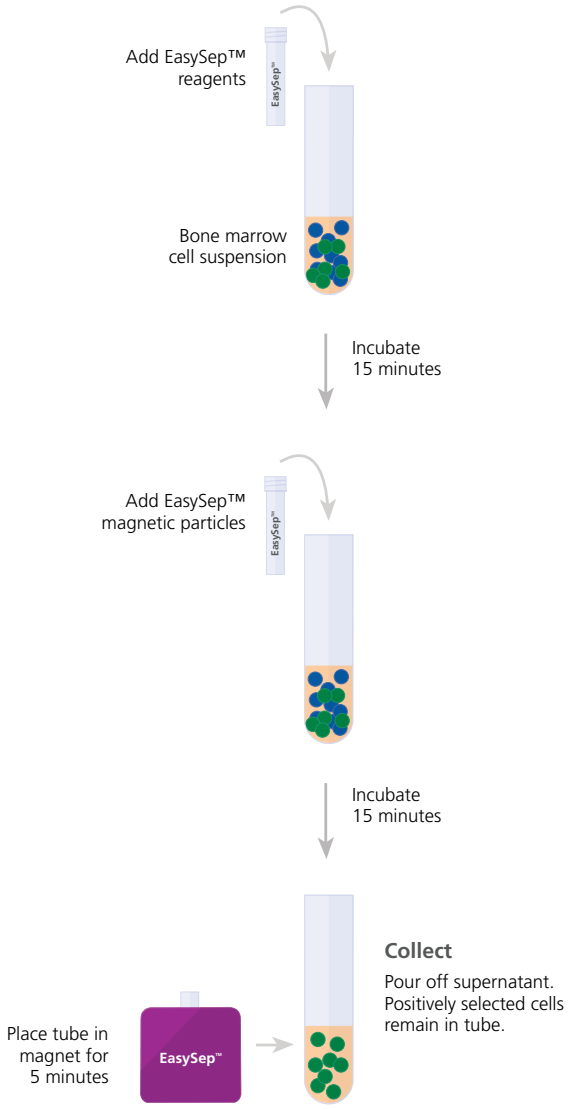

Figure 25. Overview of EasySep™ Human CD271 Positive Selection Kit II Procedure

iCell® Mesenchymal Stem Cells

iCell® Mesenchymal Stem Cells* (Catalog #70922) are human iPS cell-derived MSCs suited for basic research and regenerative biology applications. These cells express cell surface markers CD105, CD73, and CD44, exhibit classic MSC morphology, and are capable of forming colonies. iCell® Mesenchymal Stem Cells exhibit functional characteristics similar to primary MSCs including the trilineage differentiation potential.

Stromal Cells/Stromal Cells Derived in ACF Medium

Obtain MSCs derived from human bone marrow mononuclear cells, expanded for one passage using a serum-containing (Catalog #70022) or animal component-free (Catalog #70071) expansion medium**. These stromal cells are cryopreserved in Cryostor® CS10 and show great expansion capacities while maintaining robust trilineage differentiation potential in vitro.

Whole Bone Marrow

Whole bone marrow mononuclear cells (Catalog #70502)** are obtained by density gradient centrifugation of human whole bone marrow and cryopreserved in Cryostor® CS10. These mononuclear cells can be used for the enumeration of MSCs using the CFU-F assay, expansion, and/or trilineage differentiation using MesenCult™ media.

*Developed and manufactured by FUJIFILM Cellular Dynamics, Inc.

**Availability is subject to regional restrictions.

Mouse MSC Sourcing and Isolation

EasySep™ Mouse Mesenchymal Stem/ Progenitor Cell Enrichment Kit

The EasySep™ Mouse Mesenchymal Stem/Progenitor Cell Enrichment Kit (Catalog #19771) is a column-free method to isolate MSCs from mouse compact bone by negative selection. The desired cells are enriched by 50- to 200-fold using targeted antibody complexes and EasySep™ magnetic separation, enabling removal of the non-MSC cells ($CD45^+/TER119^+$).

Product Information

Human Mesenchymal Stromal Cell Research Products

PRODUCT	CATALOG #	COMPONENTS/SIZE	APPLICATIONS
STEMdiff™ Mesenchymal Progenitor Kit*	05240	• STEMdiff™-ACF Mesenchymal Induction Medium (100 mL) • Animal Component-Free Cell Attachment Substrate (1 mL) • MesenCult™-ACF Plus 500X Supplement (1 mL) • MesenCult™-ACF Plus Medium (500 mL)	Differentiation and expansion of MSCs from hPSCs
MesenCult™-ACF Plus Medium/ Culture Kit*	05445/48	• MesenCult™-ACF Plus Medium (500 mL) • MesenCult™-ACF Plus 500X Supplement (1 mL) • Animal Component-Free Cell Attachment Substrate (1 mL; available with 05448 only)	Derivation and culture of MSCs from various tissues
MesenCult™-hPL Medium Kit	05439	• MesenCult™-hPL Basal Medium (450 mL) • MesenCult™-hPL 10X Supplement (50 mL)	
MesenCult™ Proliferation Kit (Human)	05411	• MesenCult™ MSC Basal Medium (Human; 450 mL) • MesenCult™ MSC Stimulatory Supplements (Human; 50 mL)	
Animal Component-Free Cell Dissociation Kit	05426	• ACF Enzymatic Dissociation Solution (250 mL) • ACF Enzyme Inhibition Solution (250 mL)	Dissociation and passaging of human stem and progenitor cells
MesenCult™-ACF Freezing Medium	05490	MesenCult™-ACF Freezing Medium (50mL)	Cryopreservation of MSCs
MesenCult™ Adipogenic Differentiation Kit (Human)	05412	• MesenCult™ MSC Basal Medium (Human; 225 mL) • MesenCult™ 10X Adipogenic Differentiation Supplement (Human; 25 mL) • MesenCult™ 500X Adipogenic Differentiation Supplement (Human; 0.5 mL)	In vitro differentiation of MSCs into adipogenic lineage cells

Human Mesenchymal Stromal Cell Research Products (Continued)

PRODUCT	CATALOG #	COMPONENTS	APPLICATIONS
MesenCult™ Osteogenic Differentiation Kit (Human)*	05465	• MesenCult™ Osteogenic Differentiation Basal Medium (Human; 200 mL) • MesenCult™ Osteogenic Differentiation 5X supplement (Human; 50 mL)	In vitro differentiation of MSCs into osteogenic lineage cells
MesenCult™-ACF Chondrogenic Differentiation Kit	05455	• MesenCult™-ACF Chondrogenic Differentiation Basal Medium (95 mL) • MesenCult™-ACF 20X Chondrogenic Differentiation Supplement (5 mL)	In vitro differentiation of MSCs into chondrogenic lineage cells
RosetteSep™ Human Mesenchymal Stem Cell Enrichment Cocktail	15128/68	Human Mesenchymal Cell Enrichment Cocktail (2 mL/10 mL)	MSC isolation from bone marrow
EasySep™ Human CD271 Positive Selection Kit II**	17849	• EasySep™ Human CD271 Positive Selection Cocktail II (1 mL) • EasySep™ Dextran RapidSpheres™ 50100 (1mL) • Anti-Human CD32 (Fc gamma RII) Blocker, for Positive Selection (1 mL)	
iCell® Mesenchymal Stem Cells	70922	iPS cell-derived MSCs (1.0 x 10^6 viable cells)	iPS cell-derived MSC source for a variety of cellular applications
Stromal Cells/Stromal Cells Derived in ACF Medium***	70022/71	7.5 x 10^5 cultured cells derived and passaged once in serum-containing or ACF media	Primary MSC source for a variety of cellular applications
Whole Bone Marrow***	70502	≥ 100 mL bone marrow collected from adult donors using heparin as the anticoagulant	

MSC: Mesenchymal stromal cell
ACF: Animal component-free
hPSC: Human pluripotent stem cell
hPL: Human platelet lysate
iPS: Induced pluripotent stem

*Requires addition of L-Glutamine

**An EasySep™ magnet is required for this kit

***Availability is subject to regional restrictions

Mouse Mesenchymal Stromal Cell Research Products

PRODUCT	CATALOG #	COMPONENTS	APPLICATIONS
MesenCult™ Expansion Kit (Mouse)*	05513	• MesenCult™ Basal Medium (Mouse; 450 mL) • MesenCult™ 10X Supplement (Mouse; 50 mL) • MesenPure™ (0.5 mL)	Derivation and culture of mouse MSCs and MEFs
MesenCult™ Adipogenic Differentiation Kit (Mouse)*	05507	• MesenCult™ MSC Basal Medium (Mouse; 200 mL) • MesenCult™ Adipogenic Differentiation 10X Supplement (Mouse; 22 mL)	In vitro differentiation of mouse MSCs and MEFs into adipogenic lineage cells
MesenCult™ Osteogenic Stimulatory Kit (Mouse)	05504	• MesenCult™ MSC Basal Medium (Mouse; 200 mL) • MesenCult™ Osteogenic Stimulatory Supplement (Mouse; 50 mL)	In vitro differentiation of mouse MSCs and MEFs into osteogenic lineage cells
EasySep™ Mouse Mesenchymal Stem/Progenitor Cell Enrichment Kit**	19771	• EasySep™ Mouse Mesenchymal Progenitor Enrichment Cocktail (0.4 mL) • EasySep™ Biotin Selection Cocktail (2 mL) • EasySep™ D Magnetic Particles (2 mL)	Mouse MSC isolation from compact bone

MSC: Mesenchymal stromal cell

MEF: Mouse embryonic fibroblast

*Requires addition of L-Glutamine

**An EasySep™ magnet is required for this kit

Support Products

Devices

PRODUCT	CATALOG #	APPLICATIONS
Hypoxia Incubator Chamber	27310	Generates a hypoxic environment that mimics physiological conditions
Single Flow Meter	27311	Controls gas flow to generate a hypoxic environment for tissue culture in the Hypoxia Incubator Chamber

Relevant Antibodies

PRODUCT	CATALOG #
Anti-Human CD90 Antibody, Clone 5E10	60045
Anti-Human CD73 (Ecto-5'-Nucleotidase) Antibody, Clone AD2	60044
Anti-Human CD34 Antibody, Clone 581	60013
Anti-Human CD45 Antibody, Clone HI30	60018
Anti-Mouse Sca1 Antibody, Clone E13-161.7	60032
Anti-Mouse CD45 Antibody, Clone 30-F11	60030

Reagents, Kits, and Consumables

PRODUCT	CATALOG #	SIZE	APPLICATIONS
mTeSR™1	85850/57	500 mL/1	cGMP feeder-free cell culture medium for hPSCs
TeSR™-E8™	05990	1 Kit	Feeder-free, animal component-free culture medium for hPSCs
Vitronectin XF™	07180	2 mL	Xeno-free cell culture matrix that supports the growth and differentiation of hPSCs
ALDEFLUOR™ Kit	01700	1 Kit (40 tests)	Detection of viable stem cells for isolation and characterization
Lymphoprep™	07801/51	250 mL/500 mL	Density gradient medium recommended for the isolation of mononuclear cells from peripheral blood, cord blood, and bone marrow
Ammonium Chloride Solution	07800/50	100 mL/500 mL	Lysis of bone marrow red blood cells

Reagents, Kits, and Consumables (Continued)

PRODUCT	CATALOG #	SIZE	APPLICATIONS
L-Glutamine	07100	100 mL	Cell culture supplement
Costar® 6-Well Flat-Bottom Plate, Tissue Culture-Treated	38015	50 plates	Culture of anchorage-dependent cells
Tissue Culture-Treated Dishes, 100 mm	27125/27	10/240 plates	
3% Acetic Acid with Methylene Blue	07060	100 mL	Nucleated mammalian cell count
Trypan Blue	07050	100 mL	Viable cell count
D-PBS (Without Ca++ and Mg++)/D-PBS, 10X Concentrate (Without Ca++ and Mg++)	37350/54	500 mL/500 mL	Various cell culture applications
Dulbecco's Phosphate Buffered Saline with 2% Fetal Bovine Serum	07905	500 mL	
Trypsin-EDTA (0.25%/0.05%)	07901/10	500 mL/500 mL	Cell dissociation and detachment

cGMP: Current good manufacturing practice

hPSCs: Human pluripotent stem cells

D-PBS: Dulbecco's Phosphate buffered saline

FBS: Fetal bovine serum

References

1. Caroti CM et al. (2017). A novel technique for accelerated culture of murine mesenchymal stem cells that allows for sustained multipotency. Sci Rep 7(1): 1–17.
2. Baustian C et al (2015). Isolation, selection and culture methods to enhance clonogenicity of mouse bone marrow derived mesenchymal stromal cell precursors. Stem Cell Res & Ther 6 (151).
2. Quirici N et al. (2002) Isolation of bone marrow mesenchymal stem cells by anti-nerve growth factor receptor antibodies. Exp Hematol 30(7): 783–91.
3. Buhring HJ et al. (2007) Novel markers for the prospective isolation of human MSC. Ann N Y Acad Sci 1106: 262–71.

MESENCHYMAL STROMAL CELLS

Products for Your Research

STEMCELL™ TECHNOLOGIES

TOLL FREE PHONE 1 800 667 0322
PHONE +1 604 877 0713
INFO@STEMCELL.COM
TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT WWW.STEMCELL.COM

DOCUMENT #27152 VERSION 1.0.0 MAR 2019