



The next revolution in MSC culture

STEMPRO® MSC SFM
Serum-Free Human Mesenchymal Stem Cell
Culture Medium





Specially formulated for serum-free growth and expansion of human mesenchymal stem cells

STEMPRO[®] MSC SFM

- Superior efficiency of human mesenchymal stem cell (hMSC) expansion at high cell densities—less medium, surface area, and time
- Better-quality cells with primitive phenotype, retained hMSC surface marker expression, normal gene expression profiles, and retained CFU-F and trilineage mesoderm differentiation potential after five passages
- Batch-to-batch consistency, produced under cGMP and qualified using an hMSC performance assay
- Little or no adaptation required from serum-supplemented media

The first serum-free medium for hMSC culture

Daily advances in hMSC research are shedding more light on how these bone marrow–derived, nonhematopoietic stem cells may regenerate mesenchymal tissues such as bone, cartilage, muscle, ligament, tendon, and adipose. Human MSCs can overcome immune rejection associated with allogeneic transplantation, where MSC-mediated immunosuppression can reduce the incidence and severity of graft-versus-host disease (GVHD).^{1,2}

It is estimated that hMSCs comprise just 0.0001–0.01% of total bone marrow nucleated cells. As a result, these cells require robust *in vitro* cell culture expansion to obtain sufficient numbers for basic research and clinical applications. Historically, hMSC culture media comprise a basal medium (i.e., DMEM) supplemented with 10–20% fetal bovine serum (FBS), with or without additional growth factors. Although FBS does have a proliferative effect on

most cells, it also contains a number of components that are variable in concentration from one lot to the next. Some serum components are not even known or characterized. Using relatively large concentrations of FBS in hMSC culture media is undesirable for potential downstream therapeutic applications, causes inconsistent lot-to-lot performance, results in loss of differentiation potential, and wastes time trying to identify lots of preapproved MSC-Qualified FBS.

Invitrogen has developed STEMPRO[®] MSC SFM,³ a breakthrough solution that enables serum-free culture of hMSCs. STEMPRO[®] MSC SFM is a cGMP-manufactured medium that gives you the quality and consistency needed to optimize your hMSC culture. This first-in-class medium will set the stage for serum-free hMSC culture and provide a necessary research tool for the biological understanding of hMSCs.

Superior hMSC expansion at high cell densities

As hMSCs comprise a very small fraction of total bone marrow cells, expansion is critical to generate enough cells to study differentiation pathways and explore the clinical applications of hMSCs. In addition, hMSCs can be passaged only a limited number of times, thereafter experiencing reduced proliferation and differentiation potential. Hence the need to maximize the total hMSC yield per passage is essential.

Superior hMSC expansion is achieved using STEMPRO® MSC SFM compared to a classical medium (DMEM plus 10% MSC-Qualified FBS) (Figure 1). Furthermore, hMSCs grown in STEMPRO® MSC SFM exhibit superior net cell yield per passage compared to

the classical medium, so hMSCs can be grown at high cell seeding densities ($\geq 10,000$ cells/cm², and can be harvested as high as 100,000 cells/cm²), have shorter doubling times, and allow higher split ratios with similar passaging frequencies compared to control (Table 1).

Rapid and efficient cell expansion is an essential characteristic of hMSC culture media. STEMPRO® MSC SFM enables scale-up of hMSCs from 10⁶ to 10⁹ cells using 85% less medium, 92% less surface area, 16% less time, 25% fewer passages, and less effort (fewer plates and passaging and no FBS prequalification) compared to control (Table 2).

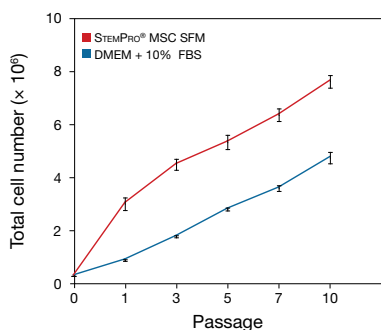


Figure 1—hMSCs grown on CELLstart™ substrate-coated dishes in STEMPRO® MSC SFM exhibit a 166% improvement in expansion over 10 passages compared to classical medium. Average net total cell number per T25 flask was calculated for hMSCs growing in STEMPRO® MSC SFM and classical medium (n = 3). The culture had a seed density of 1×10^4 cells/cm², a split frequency of 3 days, and a medium change every 2 days.

Table 1—STEMPRO® MSC SFM allows higher seeding densities and split ratios with similar passaging frequencies for MSCs versus control.

	STEMPRO® MSC SFM	Control (DMEM + 10% MSC-Qualified FBS)
Seeding density	$\geq 10,000$ cells/cm ²	$\geq 3,000$ cells/cm ²
Harvest density	100,000 cells/cm ²	20,000 cells/cm ²
Passaging frequency	7 days	7 days
Split ratio during passaging	1:10	1:7
Medium change frequency	Every 2 days	Every 2 days
Medium required	0.2 ml/cm ²	0.2 ml/cm ²

Table 2—Theoretical scale-up of MSCs from 10⁶ to 10⁹ cells using STEMPRO® MSC SFM requires less medium, surface area, passages, and time versus control.

	STEMPRO® MSC SFM	Control (DMEM + 10% MSC-Qualified FBS)
Medium required	~9 L	~61 L
Time	21 days	25 days
Total surface area	11,100 cm ²	133,333 cm ²

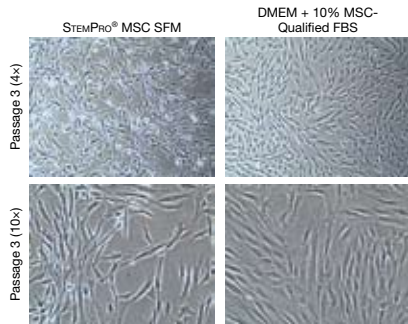


Figure 2—hMSCs grown in STEMPro[®] MSC SFM exhibit a less flattened, spindle-shaped morphology. Human MSCs expanded in STEMPro[®] MSC SFM or classical medium.

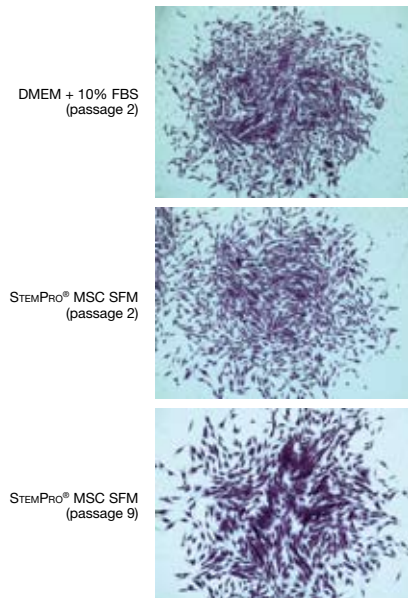


Figure 3—hMSCs grown in STEMPro[®] MSC SFM possess equal CFU-F capabilities at early (P2) and late (P9) passages. hMSCs cultured in STEMPro[®] MSC SFM (passages 2 and 9) and classical medium (passage 2) were plated to determine the frequency of CFU-F. In the CFU-F assay, cells are plated in DMEM + 10% MSC-Qualified FBS at a clonal density of 150 cells per 100 mm dish. Dishes are incubated at 37°C, 5% CO₂ in humidified air for 14 days. Plates are then rinsed and stained with 0.5% crystal violet and colonies counted using a dissection microscope.

Better-quality hMSCs with primitive morphology

hMSCs grown in STEMPro[®] MSC SFM exhibit a distinct morphology compared to those grown in control culture conditions (Figure 2). While hMSCs grown in classical medium have a flattened cell morphology and reach confluency between 1.0×10^4 and 3.0×10^4 cells/cm², hMSCs grown in STEMPro[®] MSC SFM have a much smaller, spindle-shaped morphology and can reach densities greater than 1.0×10^5 cells/cm². hMSCs displaying the latter morphology indicate a more primitive state, while wide, flattened cells suggest a later progenitor phenotype.⁴

To verify that normal phenotypes are retained by hMSCs cultured in STEMPro[®] MSC SFM, positive and negative cell surface markers, as identified by the International Society for Cellular Therapy,⁵ were characterized. Characterization of these cell surface markers by flow cytometry and real-time qRT-PCR (Table 3) showed strong hMSC marker expression. Further verifying that STEMPro[®] MSC SFM can support a primitive hMSC morphology, hMSCs grown in STEMPro[®] MSC SFM exhibit enhanced expression of the type VI intermediate filament nestin (a progenitor marker) compared to control.

The ability of STEMPro[®] MSC SFM to expand higher-quality hMSCs was also validated using the colony forming unit–fibroblast (CFU-F) assay, used to enumerate MSCs in a heterogeneous population of mesenchymal cells. Early- (passage 2) and late-passage (passage 9) hMSCs cultured in STEMPro[®] MSC SFM showed a similar CFU-F potential as compared to early-passage (passage 2) hMSCs grown in classical medium (Figure 3).

Table 3—hMSCs grown in STEMPro[®] MSC SFM display characteristic surface antigens. Flow cytometry and real-time qRT-PCR analysis of hMSCs grown in STEMPro[®] MSC SFM (passages 3 and 4, respectively*) shows expression of positive markers and no expression of negative markers. "% Relative expression" is a comparison between gene expression of hMSCs grown in STEMPro[®] MSC SFM and of hMSCs grown in classical medium.

Target	% Relative expression (vs. DMEM + 10% FBS) (qRT-PCR)	% Positive (flow cytometry)
Negative human MSC markers		
CD11b	Not tested	0.45
CD14	Not detected	Not tested
CD19	Not detected	Not tested
CD34	Not detected	1.19
CD45	Not detected	2.68
CD79a	Not tested	0.57
Positive human MSC markers		
CD44	Not tested	99.84
CD73	197.93	99.28
CD90	117.28	99.61
CD105	57.04	98.85
Nestin	4,149.89	Not tested

* Flow cytometry passage 3, RT-qPCR passage 4

Maintaining trilineage mesoderm differentiation potential

Another test of the ability of STEMPRO® MSC SFM to generate superior hMSCs was to investigate retention of trilineage mesoderm differentiation potential (adipocyte, chondrocyte, and osteoblast) over multiple passages. hMSCs cultured in STEMPRO® MSC SFM

retained this differentiation potential after 5 passages (Figure 4). In addition, hMSCs expanded in STEMPRO® MSC SFM exhibit enhanced chondrogenic differentiation potential compared to hMSCs grown in classical medium (Figure 5).

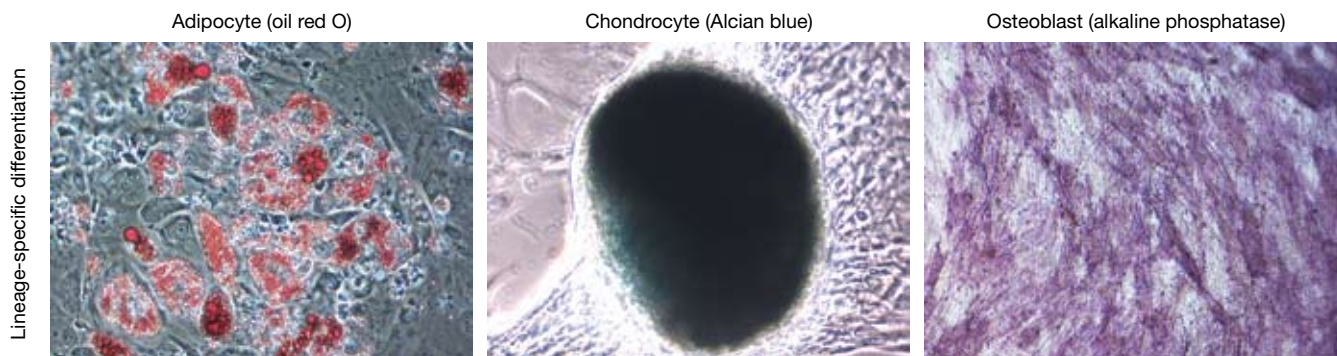


Figure 4—hMSCs cultured in STEMPRO® MSC SFM retain trilineage differentiation potential through long-term passaging. hMSCs cultured in STEMPRO® MSC SFM (after passage 5) were seeded into adipogenic, chondrogenic, or osteogenic differentiation medium for 14 days, revealing adipocytes (oil red O lipid stain), chondrocytes (Alcian blue glycosaminoglycan stain), and osteoblasts (alkaline phosphatase stain).

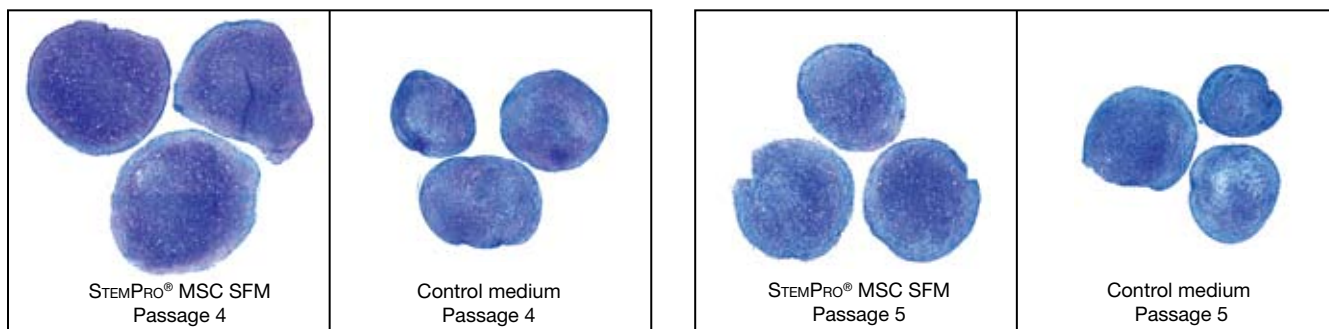


Figure 5—hMSCs propagated in STEMPRO® MSC SFM exhibit retained and enhanced chondrogenesis. Passage 4 and 5 hMSCs cultured in STEMPRO® MSC SFM or classical medium were seeded into micromass (pellet) chondrogenic differentiation culture conditions for 21 days, then stained with toluidine blue. The distinct deep purple coloration indicates the presence of chondrocytes producing proteoglycans. Data provided by L. Solchaga, Case Western Reserve University.



Preserving the gene expression profile of hMSCs

It is important that a new hMSC culture medium does not significantly alter the gene expression profile of these cells. To confirm that STEMPro[®] MSC SFM maintains standard gene expression profiles, Illumina BeadArray[™] technology was used to compare hMSCs grown in STEMPro[®] MSC SFM with those grown in classical medium. Similar gene expression is obtained when hMSCs are propagated in either medium (Figure 6).

Supporting hMSC expansion from primary bone marrow

STEMPro[®] MSC SFM is suitable not only for culturing hMSCs that have been initiated using standard adherent isolation and growth conditions (i.e., DMEM + 10% MSC-Qualified FBS) but also for hMSC expansion from primary human bone marrow. Primary bone marrow mononuclear cells seeded into T75 flasks in STEMPro[®] MSC SFM revealed continual expansion potential and enhanced proliferation of adherent cells compared to cells seeded in classical medium (Figure 7).

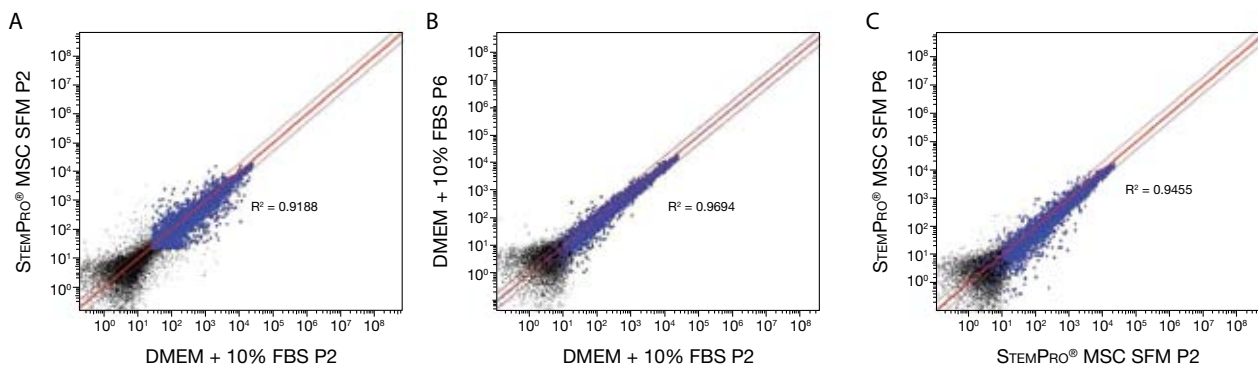


Figure 6—STEMPro[®] MSC SFM maintains global mRNA expression of hMSCs. Scatter plot analysis demonstrated that the transcriptional profile of hMSCs maintained in STEMPro[®] MSC SFM was similar to hMSCs maintained in classical medium (A), and was not substantially altered by expanding the hMSCs for 6 passages in either classical medium (B) or STEMPro[®] MSC SFM (C). Genes detected with confidence of $P > 0.01$ are represented as blue dots, and correlation values (R^2) are shown.

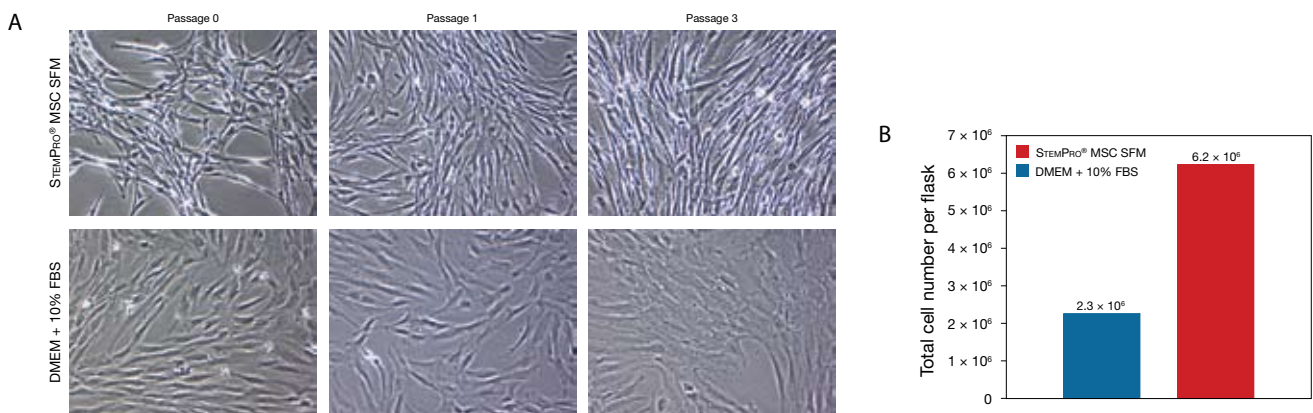


Figure 7—STEMPro[®] MSC SFM provides 270% improvement in hMSC expansion from primary bone marrow over 8 days compared to classical medium. To promote expansion of hMSCs from primary bone marrow, 20×10^6 bone marrow mononuclear cells were seeded into T75 flasks in STEMPro[®] MSC SFM. (A) Cells expanded from primary cultures displayed distinct spindle-shaped morphology and revealed continued expansion potential. (B) After 8 days in culture, cells seeded in STEMPro[®] MSC SFM revealed enhanced proliferation of adherent cells compared to an equivalent number seeded into classical medium.

CELLstart™
Defined, humanized
substrate (xeno-free)
for cell culture



CELLstart™ substrate is required to ensure hMSC attachment when growing hMSCs in the serum-free medium, STEMPro® MSC SFM

To learn more, visit www.invitrogen.com/stemcell/cellstart.

Ordering information

Product	Quantity	Cat. no.
STEMPro® MSC SFM*	1 kit	A10332-01
CELLstart™ Humanized Substrate for Cell Culture	2 ml	A10142-01

*STEMPro® MSC SFM is shipped in two parts with separate storage requirements. These components are not sold individually:

STEMPro® MSC SFM Basal Medium (500 ml; store in the dark at 2 to 8°C)

STEMPro® MSC SFM Supplement (75 ml; store in the dark at -5 to -20°C)



Advance your hMSC research with the first serum-free medium for hMSC culture.

To learn more, ask your Invitrogen Account Manager or visit www.invitrogen.com/stempro/msc for product information and protocols.

References

1. Chamberlain, G. et al. (2007) Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. *Stem Cells* 25(11): 2739–2749.
2. Aggarwal, S. et al. (2005) Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 105: 1815–1822.
3. Ng, F. et al. (2008) PDGF, TGF- β and FGF signaling is important for differentiation and growth of mesenchymal stem cells (MSCs): transcriptional profiling can identify markers and signaling pathways important in differentiation of MSC into adipogenic, chondrogenic and osteogenic lineages. *Blood* (prepublished online March 10, 2008; DOI 10.1182/blood-2007-07-103697).
4. Sekiya, I. et al. (2002) Expansion of human adult stem cells from bone marrow stroma: conditions that maximize the yields of early progenitors and evaluate their quality. *Stem Cells* 20(6): 530–541.
5. Dominici, M. et al. (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8: 315–317.



www.invitrogen.com