Opening doors to new possibilities

Key technologies for MSC research

gibco® | invitrogen™
by life technologies™

Key technologies for mesenchymal stem cell (MSC) research

Innovative solutions for the entire stem cell research workflow

Cell Culture
Isolate and Expand

Reprogramming
Engineer and Transfect

Characterization
Detection & TaqMan®

Differentiation
Growth Factors

- → Isolate, culture, and expand mesenchymal stem cells in cGMP-manufactured serum, reduced-serum, serum-free, and serum-free/xeno-free cell culture systems
- → Differentiate to your lineage of choice with our catalog of kits, growth factors, and supplements
- → Engineer mesenchymal stem cells with ease using the Neon™ Transfection System
- → Characterize mesenchymal stem cells using a wide range of primary antibodies and gene expression assay products

Advancements in mesenchymal stem cell (MSC) research are shedding light on how these stem cells may someday be used in various clinical applications such as immunomodulatory therapies (i.e., prevention of graft-versus-host disease or treatment of Crohn's disease) and in cell replacement therapies for mesenchymal tissues such as bone and cartilage [1,2].

For more than a decade, we have provided key resources to address challenges in your stem cell workflow. Designed to work together, our portfolio of stem cell products and services supports and accelerates your path to discovery.

Why is it important to use cGMP-grade reagents?

We offer the broadest portfolio of cGMP-manufactured products for MSC research. cGMP compliance ensures traceability and manufacturing reliability. Our facility in Grand Island, New York, is a medical device and *in vitro* diagnostic manufacturer. The methods and controls used in our facility for the manufacturing, processing, packaging, and storage of our products are in conformity with current Good Manufacturing Practices (cGMPs) for medical devices, 21 CFR Part 820, of the regulation. Our GIBCO® cell culture media, balanced salt solutions, and animal sera are categorized under the classification of Cell and Tissue Culture Products, class I, medical devices, *in vitro* diagnostics (21 CFR Part 864, subpart C). By following these regulatory guidelines and manufacturing under cGMP we provide high-quality products with lot-to-to consistency and traceability, helping to ensure the best foundation for reproducible, reliable results. Furthermore, since these regulatory requirements are necessary for clinical applications, using cGMP-manufactured products from the onset allows for an easy transition into the clinic.

In this overview you will find details on selected products in each of the main areas listed below. To learn more about our entire stem cell offering, and for helpful information on this topic, including protocols, we invite you to visit www.invitrogen.com/stemcell.

Contents

MSC culture product selection guide 3
MSC culture and expansion media 4
MSC culture supplements and cryopreservation 10
Extracellular matrices (ECM) and MSC passaging 11
MSC research overview 12
MSC differentiation kits and growth factors 14
MSCs and engineered MSCs 16
MSC transfection 18
MSC characterization and tracking 19
Custom culture media 22
References 23

Mesenchymal stem cell (MSC) culture product selection guide

It is estimated that human MSCs comprise just 0.0001% to 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. Today, the GIBCO® brand provides the broadest selection of cGMP-compliant complete culture systems for mesenchymal stem cells, many of which are free of animal-derived components (Table 1). These media are designed to minimize adaptation time, maximize cell performance, and meet regulatory requirements (Table 2).

Table 1. Choosing the right GIBCO® MSC culture systems for your research needs.

	Serum classical media**	Reduced-serum media**	Serum-free media**	Xeno-free media
Supports MSC derivation from primary tissue	■	■	■	■*
Maintains MSC phenotype	■	■	■	■
Supports growth at high cell density		■	■	■
Supports trilineage differentiation	■	■	■	■
Enhanced chondrogenesis		■	■	■
cGMP-manufactured, providing reliability and traceability	■	■	■	■
Lot-to-lot consistency		■	■	■
Free of animal components				■

* Requires supplementation with a low level (i.e., 2%) of human AB serum (primary culture only), after which cells can be expanded under completely serum-free/xeno-free conditions.
** Available with components originating from BSE-free (New Zealand or Australia) countries.

Table 2. MSC culture product selection guide.

Cell or tissue source: bone marrow, adipose, cord blood, placenta

Species	Culture system (see Table 1)	Complete media		Extracellular matrices	Passaging
		Basal media	Supplements		
Human, mouse, rat, dog	Serum, classical	DMEM (low glucose)	MSC-Qualified FBS		TrypLE™, trypsin, StemPro®, Accutase®
Human, mouse, rat, sheep, goat, pig, horse	Reduced-serum	MesenPRO RS™			TrypLE™, trypsin, StemPro®, Accutase®
Human	Serum-free	StemPro® MSC SFM		CELLstart™ Fibronectin	TrypLE™, trypsin, StemPro® Accutase®
Human	Xeno-free	StemPro® MSC SFM XenoFree		CELLstart™ Fibronectin	TrypLE™

Ordering information

Product	Cat. No.
Attachment Factor	S-006-100
CELLstart™	A1014201
DMEM (low glucose)	11054-020
DMEM (low glucose with GlutaMAX™-I)	10567-014
Fibronectin human, plasma	33016-015
MSC-Qualified FBS USDA (100 mL)	12662-011
MSC-Qualified FBS USDA (500 mL)	12662-029
MSC-Qualified FBS Australia (500 mL)	12664-025
MSC-Qualified FBS New Zealand (100 mL)	12665-014
MSC-Qualified FBS New Zealand (500 mL)	12665-022

Product	Cat. No.
MSC-Qualified FBS USA (500 mL)	12763-025
MesenPRO RS™	12746-012
MesenPRO RS™ (New Zealand)	E07-1000
Minimum Essential Medium (MEM) alpha Medium (1X), liquid	32571-036
StemPro® Accutase®	A1110501
StemPro® MSC SFM	A10332-01
StemPro® MSC SFM XenoFree	A10675-01
Trypsin	25300-054
TrypLE™ Select	12563-011

StemPro® MSC SFM XenoFree

Enabling serum-free and xeno-free expansion of human MSCs

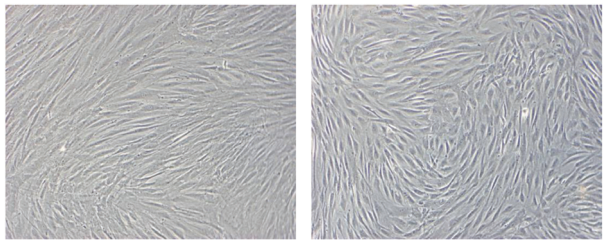
- → Maintains trilineage mesoderm differentiation potential beyond five passages (Figure 1)
- → Maintains MSC surface marker expression (Figure 1) and normal gene expression profiles
- → cGMP-manufactured serum-free and xeno-free medium for MSC expansion which ensures traceability and manufacturing reliability
- → Complete xeno-free system with CELLstart™ substrate to enable MSC attachment under serum-free conditions

Due to the low frequency of human MSCs in primary tissue, expansion of this stem cell population is critical and helps enable basic biological studies and clinical research. In addition, human MSCs can only be propagated a limited number of times, thereafter exhibiting reduced proliferation and differentiation potential. Expansion of human MSCs and adipose-derived stem cells (ADSCs) [3,4] in StemPro® MSC SFM XenoFree is comparable to classical medium (DMEM + 10% MSC-Qualified FBS) in terms of morphology and growth characteristics (Figure 2).

StemPro® MSC SFM XenoFree offers a completely animal origin–free system when used in conjunction with CELLstart™ substrate; thus cells are grown in a more physiologically similar environment that allows for more clinically relevant results.

Learn more at www.invitrogen.com/mscxenofree.

A



B

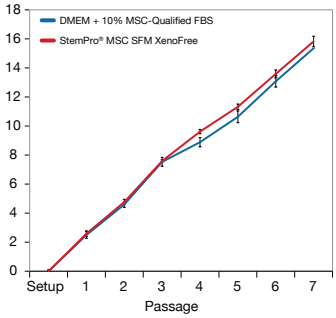
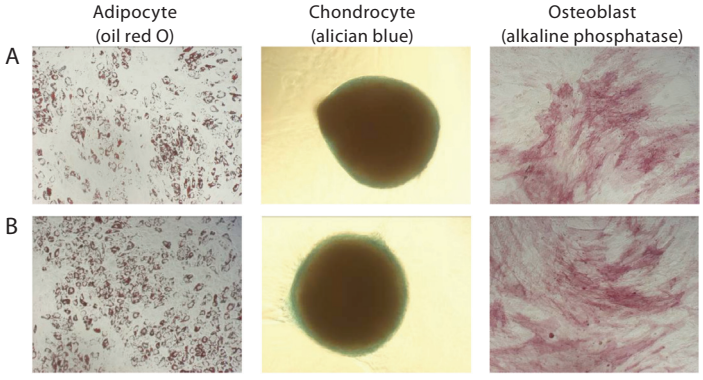


Figure 2. Human MSC expansion under xeno-free conditions. Human bone marrow–derived MSCs expanded in DMEM (low glucose) + 10% MSC-Qualified FBS, or in StemPro® MSC SFM XenoFree + CELLstart™ substrate–coated plates, revealed a similar expansion rate. **(A)** Morphology of expanded (passage 3) human MSCs (10x objective). **(B)** Net expansion of human MSCs. Input human MSCs = passage 5, 4-donor pool. Passage frequency = every 4–6 days. Seed density = 5–7 x 10^3 cells/cm^2. Harvest enzyme = TrypLE™ Express. Counting method = Countess® Automated Cell Counter.



C

DMEM + 10% MSC-Qualified FBS	
Marker	**% Positive**
CD73+/NEG-	99.7
CD90+/NEG-	99.6
CD105+/NEG-	100.0
CD34+	0.4
StemPro® MSC SFM XenoFree	
Marker	**% Positive**
CD73+/NEG-	98.2
CD90+/NEG-	99.6
CD105+/NEG-	100.0
CD34+	0.2
NEG = multiplex analysis of CD14, CD19, CD45, and HLA-DR	

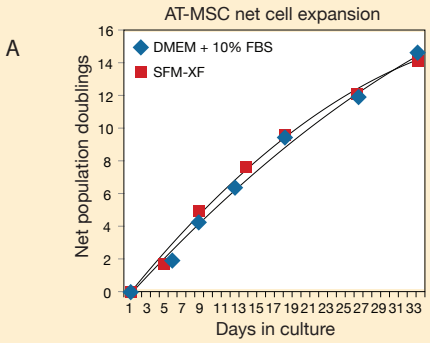
Figure 1. Characterization of human MSCs grown under xeno-free conditions. Human bone marrow–derived MSCs expanded in **(A)** DMEM (low glucose) + 10% MSC-Qualified FBS, or **(B)** StemPro® MSC SFM XenoFree + CELLstart™ substrate–coated plates, revealed a retained multilineage mesoderm differentiation potential as shown through oil red O staining (adipocyte), alcian blue staining (chondrocyte), and alkaline phosphatase staining (osteoblast). Data shown = passage 3 (input human MSCs = passage 5, 4-donor pool, 10x objective). Differentiation reagents = StemPro® Differentiation Kits (adipogenesis, chondrogenesis, osteogenesis). **(C)** Passage 5 human MSCs analyzed using multiplex flow cytometry revealed a retained characteristic human MSC surface antigen profile after expansion in classical 10% FBS-containing medium or StemPro® MSC SFM XenoFree. NEG = multiplex analysis of CD14, CD19, CD45, and HLA-DR.

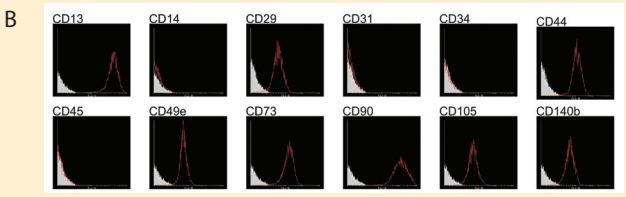
Customer testimonial

Expansion of human adipose tissue mesenchymal stem cells (AT-MSCs) in StemPro® MSC SFM XenoFree is comparable to classical medium (DMEM +10% MSC-Qualified FBS) in terms of morphology and growth characteristics (Figure 3).

- → Maintains mesoderm differentiation potential (Figure 3)
- → Maintains ADSC surface marker expression (positive: CD13, CD29, CD44, CD49e, CD73, CD90, CD105 and CD140b; negative: CD14, CD31, CD34 and CD45)

Lindolfo da Silva Meirelles, PhD
University of São Paulo–Ribeirão Preto
Center for Cell Therapy
National Institute of Science and Technology for Stem Cells and Cell Therapy
Ribeirão Preto, Brazil





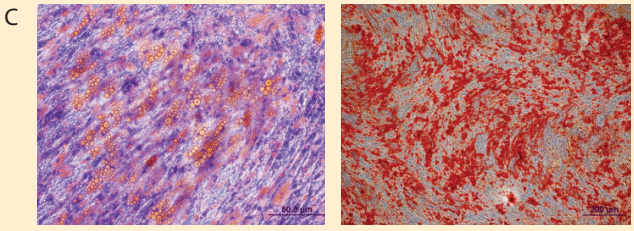
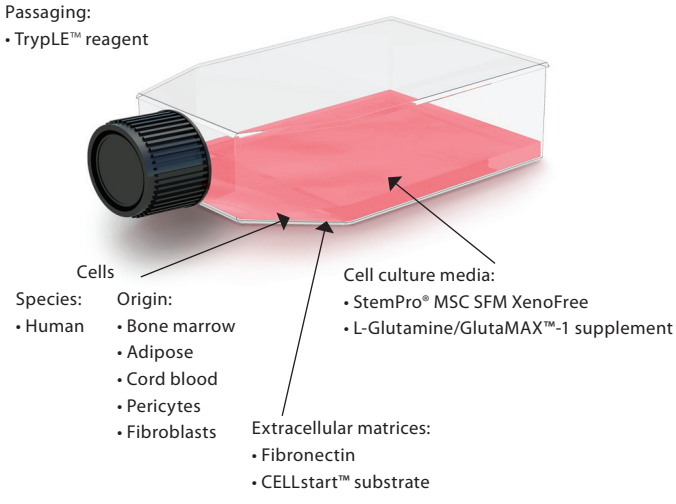


Figure 3. Expansion, differentiation, and characterization of human AT-MSCs grown under xeno-free conditions. **(A)** Human AT-MSCs expanded in classical (containing 10% FBS) medium or in StemPro® MSC SFM XenoFree on CELLstart™ substrate–coated plates revealed similar levels of cumulative cell growth (cumulative population doublings; PD). **(B)** AT-MSCs expanded in StemPro® MSC SFM XenoFree displayed a standard cell surface phenotype (passage 7). **(C)** Expanded AT-MSCs displayed retained multipotent differentiation potential, as shown through oil red O staining (adipocyte, left panel) and alizarin red S staining (osteoblast, right panel) after lineage-specific induction.



StemPro® MSC SFM XenoFree Culture System applications

- → Derivation (with additional 2% human AB serum supplementation)
- → Serum-free growth and expansion (including high-density culture)
- → Generation of mesoderm lineages
- → Growth under hypoxic conditions
- → iPSC generation [4]

Ordering information

Product	Quantity	Cat. No.
CELLstart™	2 mL	A1014201
Coating Matrix	1 kit	R011K
Fibronectin human, plasma	5 mg	33016-015
GlutaMAX™-I Supplement	100 mL	35050-061
L-Glutamine	20 mL	25030-149
StemPro® MSC SFM XenoFree	1 kit	A10675-01
TrypLE™ Select	100 mL	12563-011

StemPro® MSC Serum Free Medium (SFM)

The first serum-free medium for growth and expansion of MSCs

- → Improved expansion compared to serum-containing medium (Figure 4)
- → Maintains human MSC surface marker expression and normal gene expression profiles
- → Maintains CFU-F and trilineage mesoderm differentiation potential beyond 5 passages (Figure 6)
- → Batch-to-batch consistency and produced under cGMP, which helps ensure traceability and manufacturing reliability
- → Little or no adaptation required from classical, serum-supplemented media
- → Animal-origin components sourced from BSE-free countries (New Zealand or Australia)
- → More cells with less media, reagents, cultureware, and labor (Table 3)
- → More cells at a lower passage for more efficient differentiation

StemPro® MSC SFM [5,6,7] provides superior efficiency of human MSC expansion (Figure 4) at high cell densities, requiring less medium, surface area, and time compared with classical medium (DMEM (low glucose) + 10% FBS). While human MSCs grown in classical medium have a flattened cell morphology and reach confluency at 1.0–3.0 x 10^4 cells/cm^2, human MSCs grown in StemPro® MSC SFM have a much smaller, spindle-shaped morphology and can reach densities of >1.0 x 10^5 cells/cm^2 (Figure 5).

Visit www.invitrogen.com/stempro/msc to learn more.

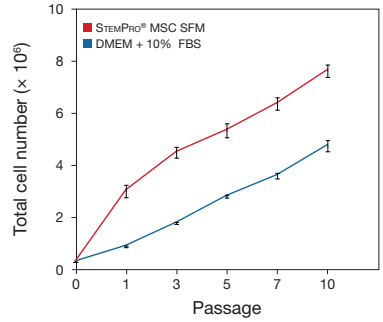


Figure 4. hMSCs grown on CELLstart™ substrate–coated dishes in StemPro® MSC SFM exhibit a 166% improvement in expansion over 10 passages, compared to classical medium. Average net total cell number per T25 flask was calculated for human MSCs growing in StemPro® MSC SFM or classical medium (n = 3). The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

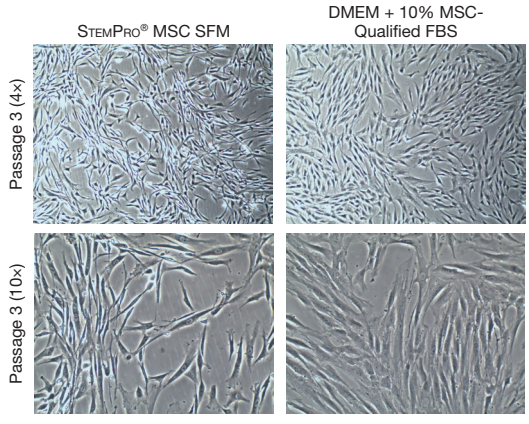


Figure 5. hMSCs grown in StemPro® MSC SFM exhibit a less flattened, spindle-shaped morphology. Human MSCs expanded in StemPro® MSC SFM or classical medium are shown.

Table 3. Benefits of StemPro® MSC SFM compared to classical media: better quality, more cells, lower cost.

	Classical media	StemPro® MSC SFM
Trilineage differentiation potential	≤passage 5	>passage 5
Time and effort	9 days at passage 3	9 days at passage 3
Cell number at passage 3	6.8 x 10^6	16.0 x 10^6
Total cost	$18.60/million cells	$17.15/million cells

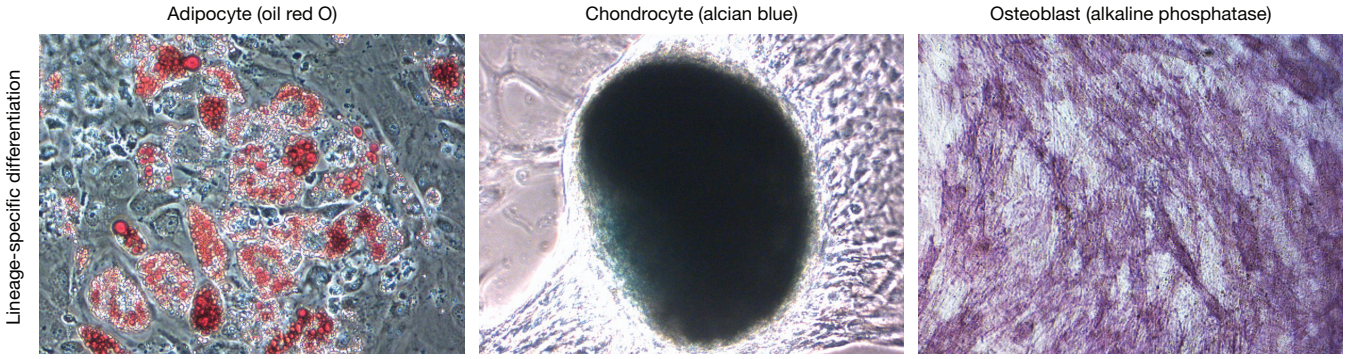
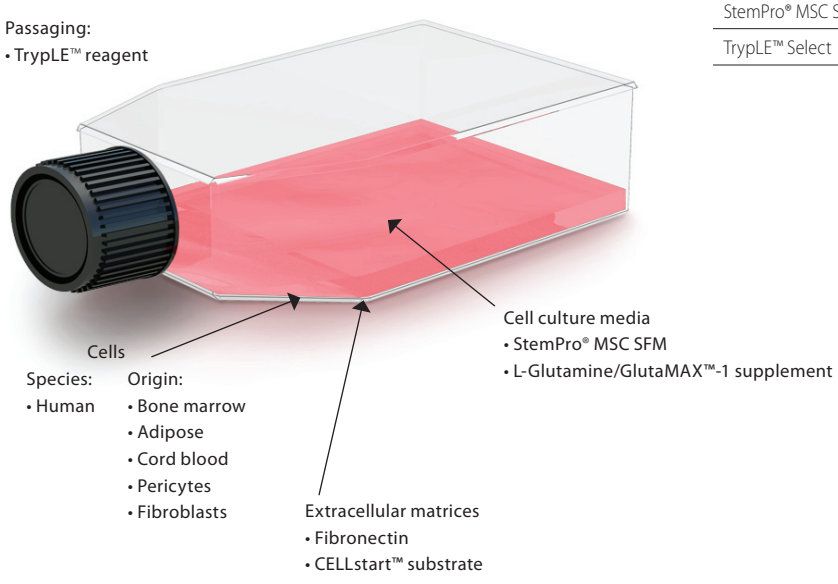


Figure 6. hMSCs cultured in StemPro® MSC SFM retain trilineage differentiation potential through long-term passaging. hMSCs cultured in StemPro® MSC SFM (after passage 5) were seeded into adipogenic, chondrogenic, or osteogenic differentiation medium for 14 days, revealing adipocytes (oil red O lipid stain), chondrocytes (alcian blue glycosaminoglycan stain), and osteoblasts (alkaline phosphatase stain).

StemPro® MSC SFM Culture System applications

- → Derivation, growth, and expansion, including high-density culture
- → Generation of mesoderm lineages



Ordering information

Product	Quantity	Cat. No.
Attachment Factor	100 mL	S-006-100
CELLstart™	2 mL	A1014201
Fibronectin Human, Plasma	5 mg	33016-015
GlutaMAX™-I Supplement	100 mL	35050-061
L-Glutamine	20 mL	25030-149
StemPro® MSC SFM	1 kit	A10332-01
TrypLE™ Select	100 mL	12563-011

MesenPRO RS™ Medium

A reduced-serum (2%) medium for MSC culture

- → Retains trilineage mesoderm differentiation capacity and supports gene expression profiles comparable to classical media
- → Contains 2% FBS, reducing the variability introduced by adding 10–20% FBS (typically used in classical media)
- → Reduces the time and money spent prequalifying FBS lots
- → Batch-to-batch consistency and produced under cGMP, which helps ensure traceability and manufacturing reliability

MesenPRO RS™ Medium [4,7,8,9,10,16] consistently improves expansion of MSCs (Figure 7) compared with classical media (DMEM + 10% FBS) and maintains trilineage mesoderm differentiation potential (Figure 8).

Visit www.invitrogen.com/mesenpro to learn more.

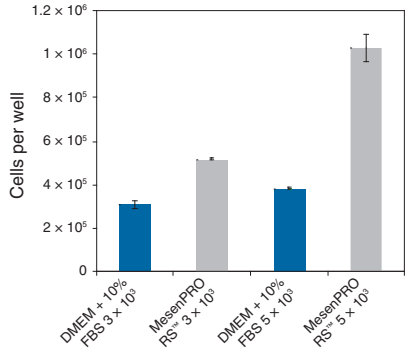


Figure 7. MesenPRO RS™ Medium provides a 69–169% improvement in expansion over 6 days, compared to classical media. MSCs were isolated from normal human bone marrow mononuclear cells by standard techniques. Early-passage cells were plated into 6-well plates at either 3 x 10^3 or 5 x 10^3 cells/cm^2 in DMEM (low glucose) containing 4 mM L-glutamine, 5 µg/mL gentamicin, and 10% MSC-Qualified FBS; or MesenPRO RS™ Medium containing 2 mM L-glutamine and 5 µg/mL gentamicin. Cells were incubated at 37°C with 5% CO_2 in humidified air and fed on day 3. On day 6, cells were harvested using TrypLE™ reagent and counted with a Z2 COULTER COUNTER particle counter (Beckman Coulter, Inc.). Data represent cell count averages from duplicate wells ($p \leq 0.007$ and $p \leq 0.002$, respectively, by Student's t-test).

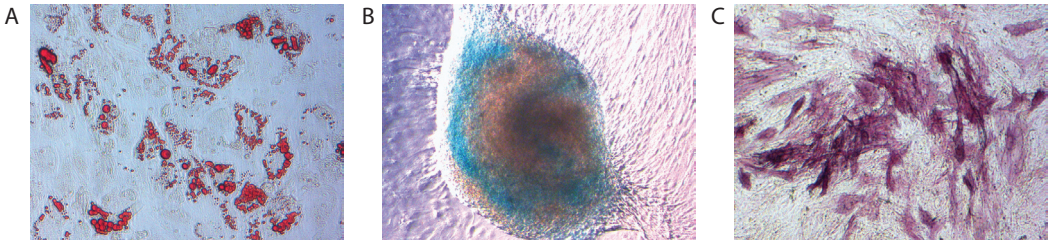
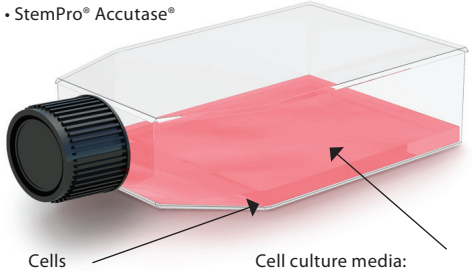


Figure 8. Human MSCs cultured in MesenPRO RS™ Medium retain trilineage differentiation potential. Human MSCs cultured in MesenPRO RS™ Medium were seeded into adipogenic, chondrogenic, or osteogenic differentiation medium for 14 days, revealing **(A)** adipocytes (oil red O lipid stain), **(B)** chondrocytes (alcian blue glycosaminoglycan stain), and **(C)** osteoblasts (alkaline phosphatase stain).

Passaging:
- TrypLE™ reagent
- Trypsin
- StemPro® Accutase®



Cells

Species:
- Human
- Mouse
- Sheep
- Pig

Origin:
- Bone marrow
- Adipose
- Umbilical cord

Cell culture media:
- MesenPRO RS™
- L-Glutamine/GlutaMAX™-I supplement

MesenPRO RS™ culture system applications

- → Derivation, growth and expansion, and generation of mesoderm lineages
- → Supports expansion in microcarrier cultures [8]

Ordering information

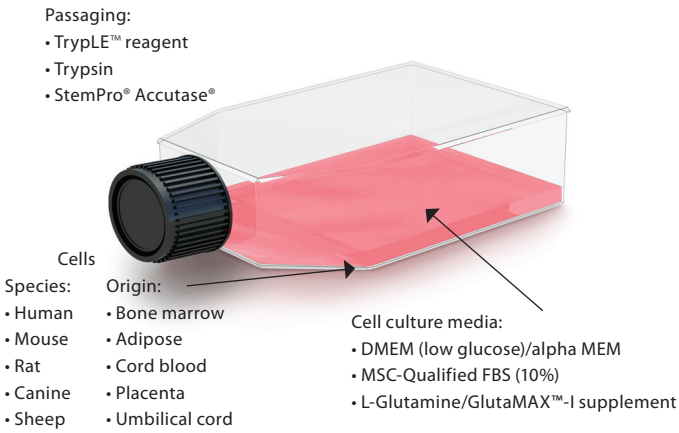
Product	Quantity	Cat. No.
GlutaMAX™-I Supplement	100 mL	35050-061
L-Glutamine	20 mL	25030-149
MesenPRO RS™	1 kit	12746-012
MesenPRO RS™ (New Zealand origin)	1 kit	E07-1000
TrypLE™ Select	100 mL	12563-011
StemPro® Accutase®	100 mL	A1110501
Trypsin	100 mL	25300-054

MSC-Qualified Fetal Bovine Serum (FBS)

- → Avoid time-consuming testing and lot-to-lot variation problems
- → Attain enhanced MSC clonal efficiency (Figure 9)
- → Improve expansion and obtain sustainable MSC differentiation (Figure 10)

FBS is a component used for the "classical" method of culturing MSCs. However, there are many unknown elements in FBS, such as signaling molecules, apoptotic factors, and nutrients. The variable concentrations of these components can cause lot-to-lot variation, which means that some FBS lots do not support MSC culture. For that reason, extensive and time-consuming pre-testing is required. Our MSC-Qualified FBS [7,11] minimizes the need for you to test multiple FBS lots to identify the optimal one for MSC research.

Visit www.invitrogen.com/stemcell/msc to learn more.



MSC-Qualified FBS–based culture system applications

- → Derivation, growth, expansion, and generation of mesoderm lineages
- → Growth under hypoxic conditions
- → Supports expansion in microcarrier cultures

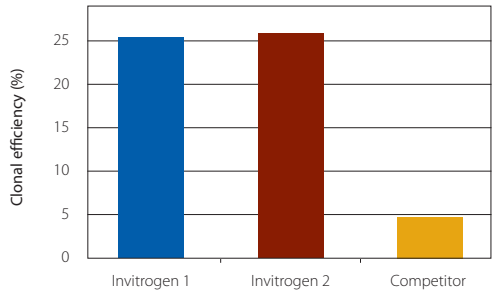


Figure 9. Effect of FBS source on MSC clonal efficiency. Mesenchymal stem cells were isolated from normal human bone marrow mononuclear cells by standard techniques. Early-passaged cells were plated into duplicate 100 mm tissue culture dishes at a seeding density of 100 cells per plate in DMEM (low glucose), 4 mM L-glutamine, 5 µg/mL gentamicin, and 10% of the indicated FBS. On day 14, the medium was removed and the plates were rinsed and stained with 0.5% crystal violet in methanol for 30 min. Plates were rinsed and dried, and the colonies were counted using a dissection microscope. Only colonies with at least 50 cells were counted. Invitrogen's MSC-Qualified FBS outperformed a competitor's MSC-qualified FBS ($P < 0.05$; Student's t-test).

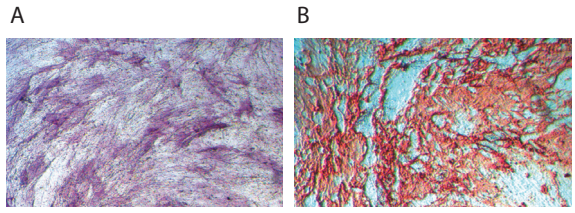


Figure 10. Histological staining of osteogenic cultures. MSCs were initiated in DMEM, 10% MSC-Qualified FBS, 4 mM L-glutamine, and 5 µg/mL gentamicin at a seeding density of 5 x 10^3 cells/cm^2 in 12-well plates. Two hours after seeding, the medium was changed and supplemented with 100 nM dexamethasone, 10 mM sodium β-glycerophosphate, 50 µM ascorbic acid-2-phosphate, and 10 ng/mL BMP-2. Plates were fed every 3–4 days. Control wells did not contain bone induction factors. **(A)** Plates were stained for alkaline phosphatase on day 14 using commercially available kits. **(B)** Plates were stained with alizarin red S on day 25 using standard staining techniques.

Ordering information

Product	Quantity	Cat. No.
Minimum Essential Medium (MEM) alpha Medium (1X), liquid	500 mL	32571-036
DMEM (low glucose)	500 mL	11054-020
GlutaMAX™-I Supplement	100 mL	35050-061
L-Glutamine	20 mL	25030-149
MSC-Qualified FBS USDA	100 mL	12662-011
MSC-Qualified FBS USDA	500 mL	12662-029

Product	Quantity	Cat. No.
MSC-Qualified FBS Australia	500 mL	12664-025
MSC-Qualified FBS New Zealand	100 mL	12665-014
MSC-Qualified FBS New Zealand	500ml	12665-022
MSC-Qualified FBS USA	500 mL	12763-025
StemPro® Accutase®	100 mL	A1110501
TrypLE™ Select	100 mL	12563-011
Trypsin	100 mL	25300-054

StemPro® LipoMAX™

Defined xeno-free lipid supplement

- → Supports MSC and adipose-derived stem cell (ADSC) expansion
- → Provides supplemental lipids and cholesterol to cultured cells
- → Improves expansion of ADSCs grown under serum-free conditions (Figure 11)
- → Processed by proprietary methods from pooled human plasma under cGMP guidelines, helping to ensure traceability and manufacturing reliability

StemPro® LipoMAX™ is a human-derived, lipoprotein-based cell culture supplement for stem cells. Lipoproteins contribute a critical role in the regulation and maintenance of cellular growth and metabolism [1,12]. Addition of StemPro® LipoMAX™ under serum-free conditions can improve expansion of ADSCs by ~50% after 3 passages.

Visit www.invitrogen.com/stemcell/msc to learn more.

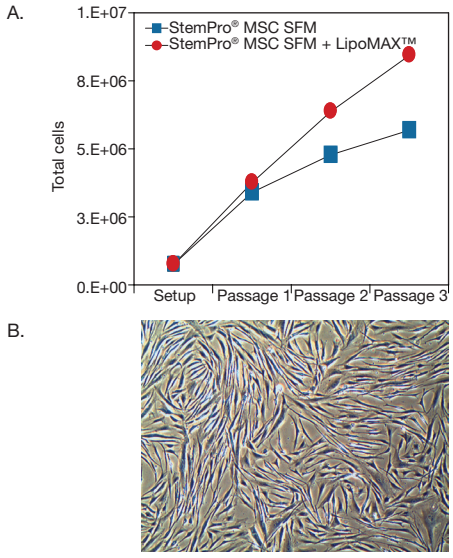


Figure 11. Enhanced growth of ADSCs in StemPro® LipoMAX™ supplement. **(A)** ADSCs expanded in StemPro® MSC SFM with 1:100 StemPro® LipoMAX™ exhibit an improvement in expansion of ~50% over 3 passages, compared to StemPro® MSC SFM alone. **(B)** Morphology of ADSCs expanded for 3 passages in StemPro® MSC SFM with StemPro® LipoMAX™ supplement.

Synth-a-Freeze®

Defined, protein-free cryopreservation medium

- → Convenient one-component cyropreservation system that can be used with any standard freezing protocol
- → Synth-a-Freeze® performs as well as standard, serum-containing cryopreservation medium for a variety of stem cell types and primary cell lines, including MSCs (Figure 12), ESCs, iPSCs, keratinocytes, fibroblasts, and epithelial and endothelial cells [13]
- → Produced under cGMP, which helps ensure traceability and manufacturing reliability

Synth-a-Freeze® is a sterile, defined liquid cryopreservation medium containing 10% dimethylsulfoxide (DMSO). Synth-a-Freeze® does not contain antibiotics, antimycotics, hormones, growth factors, serum, or proteins, and offers an easy-to-use, convenient cryopreservation system.

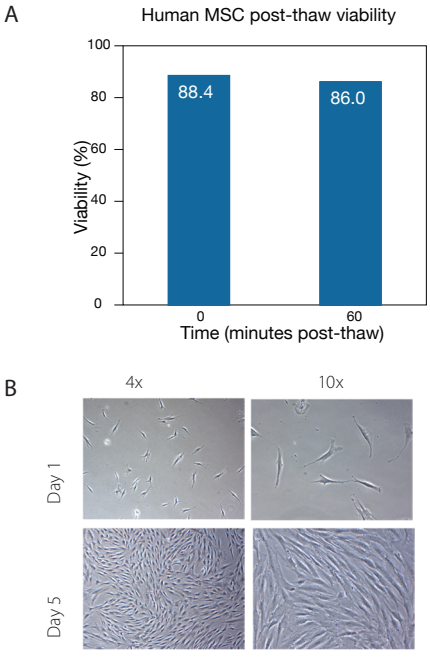


Figure 12. MSC cryopreservation in Synth-a-Freeze® medium. **(A)** MSCs were expanded in DMEM + 10% MSC-Qualified FBS and frozen in Synth-a-Freeze® medium. After thawing, percent cell viability was checked at 0 and 60 minutes after recovery. **(B)** 4x and 10x captured images of MSCs recovered from cryopreservation in Synth-a-Freeze® and expanded in DMEM + 10% MSC-Qualified FBS for 5 days.

Ordering information

Product	Quantity	Cat. No.
StemPro® LipoMAX™	5 mL	A10850-01
Synth-a-Freeze® Cryopreservation Medium	50 mL	A12542-01

CELLstart™ substrate

First xeno-free, fully defined cell culture substrate

- → Maintains multipotency, morphology, and trilineage mesoderm differentiation potential of human MSCs (Figure 13)
- → Consistent lot-to-lot performance
- → cGMP-compliant, which helps ensure traceability and manufacturing reliability

CELLstart™ substrate is the first xeno-free substrate that contains components only of human origin. CELLstart™ substrate enables attachment and serum-free expansion of human MSCs and provides the perfect substrate for applications where more physiological, *in vivo*-like conditions are desired [4].

Learn more at www.invitrogen.com/3D-cellculture.

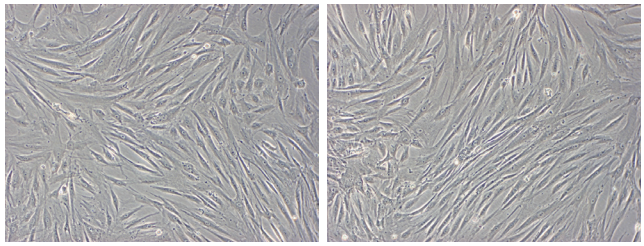


Figure 13. Multi-passage human MSC expansion on CELLstart™ substrate. Human MSCs from a 4-donor, passage 4 pool, expanded for 9 passages in StemPro® MSC SFM XenoFree on CELLstart™ substrate–coated flasks, exhibit a less flattened spindle-shaped morphology.

TrypLE™ Select

The superior replacement for trypsin

- → Gentle on cells—higher plating efficiency
- → Saves time—eliminates the need to stagger harvesting
- → Room temperature–stable—ready to use when you need it
- → Easy to use—directly substitutes into existing protocols
- → Maintains normal morphology of MSCs and surface marker expression (Figure 14)

Choose the reagent that makes cell dissociation more convenient for you and less harsh on your cells. TrypLE™ Select cell dissociation enzyme is stable at room temperature, gentle on cells, and free from any animal-derived components.

Learn more at www.invitrogen.com/trypleselect.

A

Target	% Relative expression (TrypLE™ vs. trypsin)	% Positive
Positive human MSC markers		
CD90	106.71	98.89

B

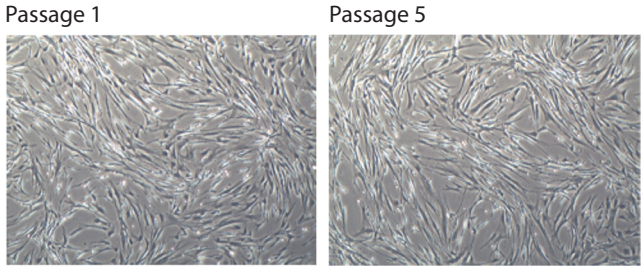


Figure 14. Human MSCs dissociated with TrypLE™ reagent over 5 passages display normal morphology and characteristic surface antigens. (A) Flow cytometry analysis of human MSCs expanded in DMEM + 10% MSC-Qualified FBS and dissociated with TrypLE™ reagent over 5 passages shows expression of positive marker CD90. "% Relative expression" is a comparison between human MSCs treated with TrypLE™ and trypsin. **(B)** Morphology of human MSCs expanded (passage 1 and 5) in StemPro® MSC SFM on CELLstart™ substrate–coated plates and dissociated with TrypLE™ reagent.

Ordering information

Product	Quantity	Cat. No.
CELLstart™	2 mL	A1014201
Fibronectin human, plasma	5 mg	33016-015
Attachment Factor	100 mL	S006100
StemPro® Accutase®	100 mL	A1110501
Trypsin	100 mL	25300-054
TrypLE™ Select	100 mL	12563-011

Mesenchymal stem cell research

Isolation

Bone marrow
- StemPro® Rat Alk Phos Expressing Mesenchymal Stem Cells
- GIBCO® Mouse (C57BL/6) Mesenchymal Stem Cells
- GIBCO® Rat (SD) Mesenchymal Stem Cells

Adipose tissue
- StemPro® Human Adipose-Derived Stem Cells

Blood
Dermis
Placenta
Umbilical cord
Deciduous teeth
Synovial fluid
Synovial membrane
Amniotic fluid
Skeletal muscle
Postnatal organs

Multilineage adult stem cells
(i.e., MAPC, MPLC, MIAMI, VSEL, etc.)

Pluripotent stem cells
ESC
iPS

Expansion

Xeno-free, serum-free and reduced-serum media:
Complete media
StemPro® MSC SFM XenoFree
StemPro® MSC SFM (serum-free)
MesenPRO RS™ (reduced-serum: 2%)

Classical human MSC media:
Basal media
DMEM (low glucose)
αMEM

Supplement
10–20% MSC-Qualified FBS (+/– growth factors)

Differentiation

StemPro® Osteogenesis Differentiation Kit

StemPro® Chondrogenesis Differentiation Kit

5-Azacytidine
Amphotericin B

Osteoblast

Chondrocyte

Myoblast fusion

Bone

Markers:
Osteocalcin (BGLAP)
Osteopontin (SPP1)
Runt-related transcripton factor 2 (RUNX2)
Phosphate-regulating neutral endopeptidase (PHEX)

Staining:
Alkaline phosphatase
Alizarin red S
von Kossa

Cartilage

Markers:
Aggrecan 1 (AGC1)
Biglycan (BGN)
Decorin (DCN)
Annexin VI (ANXA6)
Matrilin 1 (MATN1)
Matrix metalloproteinase 13 (MMP13)
Sox9
Collagen, type II, alpha 1 (COL2A1)
Cartilage oligomeric matrix protein (COMP)

Staining:
Alcian blue
Toluidine blue

Muscle

Markers:
Skeletal muscle:
Myogenic differentiation antigen 1 (MYOD1)
Paired box gene 7 (PAX7)
Myogenic factor 4 (MYOG)
Myosin light chain 1, skeletal muscle isoform (MYL1)

Cardiac muscle:
GATA binding protein 6 (GATA6)
Homeobox protein CSX (NKX2-5)
T box 5 (TBX5)
Myosin heavy chain 6, cardiac muscle, alpha (MYH6)
Atrial natriuretic factor (NPPA)
Connexin 32 (GJB1)

Smooth muscle:
GATA binding protein 4 (GATA4)
Transgelin (TAGLN)
Myosin heavy chain, smooth muscle isoform (MYH11)
Actin alpha 2 (ACTA2)
Calponin (CNN1)

See page 23 for MSC pathway references.

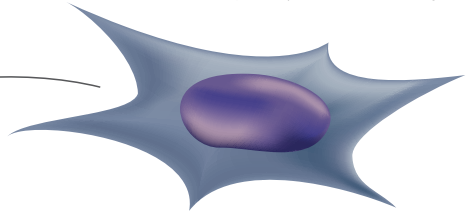
Alternative human MSC media:
Basal media
DMEM (high glucose)
DMEM/F-12
DMDM (low-glucose)/MCDB 201
IMDM
RPMI 1640

Supplement
≤10% Human serum
Human platelet-rich plasma
2% FBS + growth factors
(i.e., bFGF, EGF, PDGF, etc.)

Characterization

Chemokine receptors:
CCR1, 2, 3, 4, 7, 8, 9, 10
CXCR1, 2, 3, 4, 5, 6

Cytokine production:
Interleukins: 1α, 1b, 6, 7, 8, 11, 14, 15
Colony-stimulating factors: M-CSF, G-CSF, GM-CSF
Other hematopoietic cytokines: LIF, SCF, Flt-3 ligand, TPO



Surface markers:
Positive
CD13, CD29, CD44, CD49a-f, CD51, CD54, CD59, CD71, CD73, CD90, CD105, CD106, CD147, CD166, Stro-1, MHC I

Negative
CD11b, CD14, CD18, CD19, CD31, CD34, CD36, CD45, CD56, CD79α, CD117, MHC II, CD40, CD80, CD86

Applications

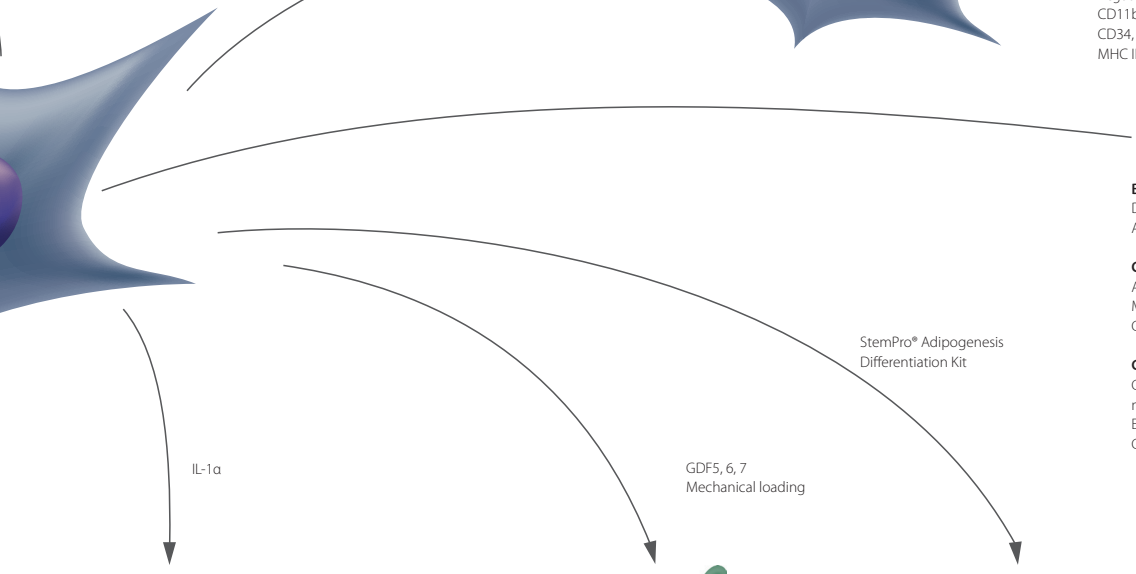
Basic biology:
Developmental studies
Animal disease models

Cancer biology:
Antitumorigenic effects
Metastatic promotion
Genetic stability

Genomics/genetic studies:
Gene expression profiling
miRNA profiling
Epigenetics
Genetic manipulation

Drug screening:
Differentiated targets
Disease mechanisms
Toxicity testing
Therapeutic screens
Stem cell signaling
Differentiation screens

Clinical trial applications:
Cell replacement therapy
Trophic support
Antiapoptotic applications
Immune modulation



IL-1α

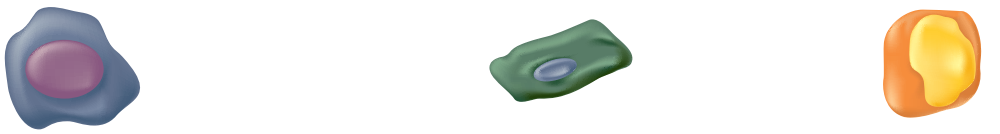
GDF5, 6, 7
Mechanical loading

StemPro® Adipogenesis Differentiation Kit

Stromal fibroblast

Tenoblast

Preadipocyte



Stroma

Markers:
Melanoma adhesion molecule (MCAM)
Fibroblast activation protein alpha (FAP)
Multimerin 2 (MMRN2)
Tumor endothelial marker 1 (CD248)

Tendon

Markers:
Collagen, type I, alpha 1 (COL1A1)
Collagen, type III, alpha 1 (COL1A3)
Tenascin C (TNC)
Scleraxis homolog B (SCXB)
Tenomodulin (TNMD)

Adipose

Markers:
Peroxisome proliferation-activated receptor gamma (PPARG)
Lipoprotein lipase (LPL)
Fatty acid binding protein 4 (FABP4)
Adiponectin (ADIPOQ)
Leptin (LEP)
Perilipin (PLIN)
Complement factor D (CFD)

Staining:
Oil red O
Nile red

MSC Differentiation Kits

Standardized protocols for human MSC differentiation

- → Reliable induction of human MSCs into adipocytes (Figure 17), chondrocytes (Figure 16), and osteoblasts (Figure 15)
- → Complement StemPro® MSC SFM XenoFree, StemPro® MSC SFM, MesenPRO RS™ Medium, and MSC-Qualified FBS–containing cell expansion systems
- → Produced under cGMP, with each lot performance-qualified using PCR
- → Reconstituted differentiation kit media (basal medium plus supplement) are stable for up to 1 month
- → Support differentiation of human, mouse, and rat MSCs

Human MSCs differentiate to adipocytes, chondrocytes, and osteoblasts under appropriate cell culture conditions [6,14,15,16]. The ISCT position article [19] used these lineages to define the trilineage mesoderm differentiation potential of human MSCs. Even though cell culture conditions used to differentiate human MSCs to adipocytes, chondrocytes, and osteocytes are well established, researchers report variable success in differentiation efficiencies, arising from quality differences in the raw materials used to generate differentiation cocktails. This issue is further compounded by the differentiation cocktails' serum requirement, which is a major source of lot-to-lot inconsistency. These kits provide researchers with all the necessary prequalified components, manufactured under cGMP to help reduce this variability.

For more information, visit www.invitrogen.com/stempro/mscdiff.

Ordering information

Product	Quantity	Cat. No.
StemPro® Adipogenesis Differentiation Kit	1 kit	A10070-01
StemPro® Chondrogenesis Differentiation Kit	1 kit	A10071-01
StemPro® Osteogenesis Differentiation Kit	1 kit	A10072-01

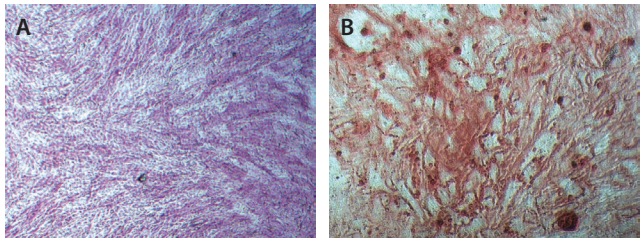


Figure 15. Osteogenesis-induced differentiation of bone marrow–derived human MSCs using the StemPro® Osteogenesis Differentiation Kit. **(A)** Human MSCs induced under osteogenic conditions for 14 days were fixed and stained with alkaline phosphatase, a marker for proliferating osteoblasts. **(B)** Human MSCs induced under osteogenic conditions for 28 days were fixed and stained with alizarin red S, a dye that specifically binds to calcium matrix formations.

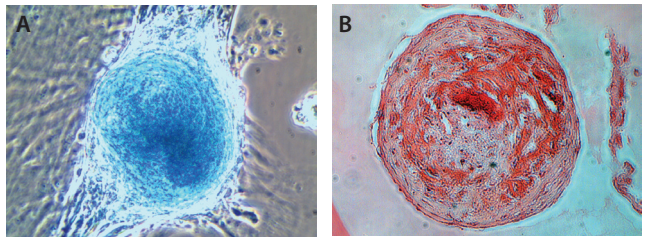


Figure 16. Chondrogenesis-induced differentiation of bone marrow–derived human MSCs using the StemPro® Chondrogenesis Differentiation Kit. **(A)** Alcian blue staining of developing chondrogenic pellet. **(B)** Safranin O staining of a cross-section of a day 20 chondrogenic pellet.

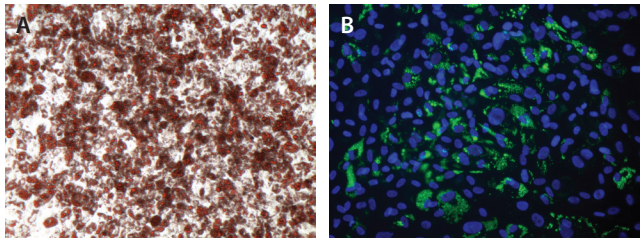


Figure 17. Adipogenesis-induced differentiation of bone marrow–derived hMSCs using the StemPro® Adipogenesis Differentiation Kit. **(A)** hMSCs were induced under adipogenic conditions for 14 days, fixed, and stained with oil red O, a marker for lipid-rich vesicles. **(B)** hMSCs were induced under adipogenic conditions for 7 days, fixed, and lipid vesicles visualized with LipidTOX™ Green neutral lipid stain (green). Hoechst 33342 was applied as a nuclear counterstain (blue).

Growth factors

GIBCO® growth factors provide the activity, stability, and validation required for your stem cell research

- → High biological activity—more results with less protein (Figure 18)
- → High purity—no interference from other proteins or contaminants (Figure 19)
- → Proven compatibility—GIBCO® proteins are bioassayed with GIBCO® media
- → Convenient access—GIBCO® proteins can be stocked in your Invitrogen Supply Center

Learn more at www.invitrogen.com/growthfactors.

GIBCO® quality assurance

To ensure that GIBCO® recombinant proteins are of the highest quality, each protein is analyzed for purity along with refolding and structural homogeneity to ensure that a biologically active protein is produced. In-house bioactivity testing includes cell proliferation, cytotaxis, calcium flux, secondary cytokine up-regulation, induction of surface antigen expression, and protease assays.

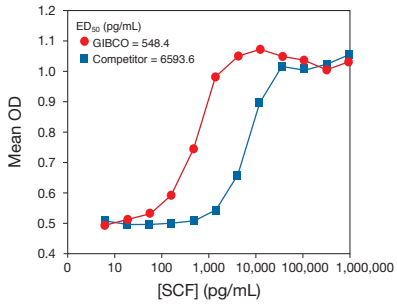


Figure 18. More bioactive protein due to exceptional refolding techniques. Proliferation of MO7e cells in response to GIBCO® SCF (PHC2115) and a competitor's product. As illustrated by the lower ED_{50}, less GIBCO® SCF is required to yield a response.

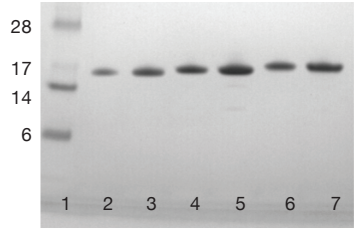


Figure 19. Gel electrophoresis to demonstrate purity of human FGF-basic protein. Lane 1: marker; lane 2: 1 µg FGF-basic (competitor); lane 3: 2 µg FGF-basic (competitor); lane 4: 1 µg FGF-basic (10–155), lane 5: 2 µg FGF-basic (10–155), Lane 6: 1 µg FGF-basic (full length), lane 7: 2 µg FGF-basic (full length).

Ordering information

Product	Quantity	Cat. No.
BMP-2 (human)	10 µg	PHC7145
BMP-4 (human)	5 µg	PHC7914
BMP-7 (active) (human)	10 µg	PHC7204
	10 µg	PHC7104
CTGF (human)	20 µg	PHG0286
EGF (human)	25 µg	PHG0315
FGF-basic (full length) (human)	10 µg	PHG0264
	25 µg	PHG0266
	100 µg	PHG0261
	1 mg	PHG0263
FGF-basic (human)	10 µg	13256-029
FGF-basic, AA 10–155 (human)	10 µg	PHG0024
	50 µg	PHG0026
	100 µg	PHG0021
	1 mg	PHG0023
Insulin (human)	5 mL	12585-014
IL-1α (human)	2 µg	PHC0014
	5 µg	PHC0015
	25 µg	PHC0017
	100 µg	PHC0011
	1 mg	PHC0013
Myelin Basic Protein (MBP) (bovine)	10 mg	13228-010
PDGF (human)	5 µg	PHG0044
	10 µg	PHG0045
	50 µg	PHG0046
	100 µg	PHG0041
	1 mg	PHG0043
TGF-α (human)	100 µg	PHG0051
TGF-β1 (human)	5 µg	PHG9204
	10ug	PHG9214
	100 µg	PHG9211
	250 µg	PHG9202
	1 mg	PHG9203
TGF-β2 (human)	5 µg	PHG9114
TGF-β3 (human)	5 µg	PHG9305
	250 µg	PHG9302

StemPro® Human Adipose-Derived Stem Cells (ADSCs)

- → ADSCs are passaged only once after isolation from human lipoaspirate tissue before cryopreservation (Figure 20)
- → After thawing and expansion, ADSCs show high purity, as demonstrated by flow cytometric analysis of positive (CD29, CD44, CD73, CD90, CD105, and CD166) and negative (CD14, CD31, CD45, and Lin1) cell-surface marker expression (Figure 21)
- → Expanded in MesenPRO RS™ Medium—a reduced-serum (2%) medium that reduces ADSC doubling times
- → Each lot of ADSCs originates from a single donor of human lipoaspirate tissue
- → ADSCs can be reprogrammed with higher efficiencies than fibroblasts, and the resulting iPSCs can be generated and maintained under feeder-free conditions [4]

Human adipose-derived stem cells (ADSCs) [17] are isolated from human lipoaspirate tissue, collected during liposuction procedures and cryopreserved from primary cultures. ADSCs have phenotypic and functional characteristics very similar to bone marrow–derived mesenchymal stem cells, and have equal potential to differentiate into cells and tissues of mesodermal origin such as adipocytes, cartilage, and bone (Figure 22).

Learn more at www.invitrogen.com/stempro/adsc.

Ordering information

Product	Quantity	Cat. No.
StemPro® Human Adipose-Derived Stem Cells	1 vial	R7788-115
StemPro® Human Adipose-Derived Stem Cell Kit	1 kit	R7788-110

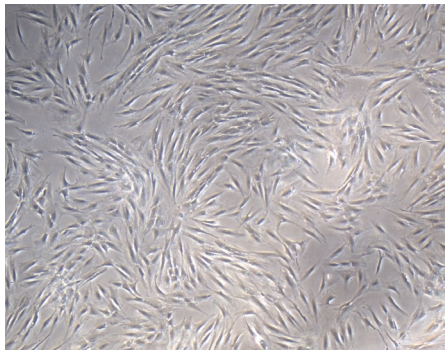


Figure 20. Human ADSCs in culture at passage 3. Phase-contrast image of human ADSCs expanded in MesenPRO RS™ Medium.

Figure 21. Phenotypic profile of ADSC cell-surface markers at passage 2. Flow cytometric analysis of ADSC cell-surface proteins at passage 2 or 3 using the following criteria: >95% for positive markers, <2% for negative markers.

Marker	>95% positive events	<2% positive events
CD14		•
CD29	•	
CD31		•
CD44	•	
CD45		•
CD73	•	
CD90	•	
CD105	•	
CD166	•	
Lin1		•

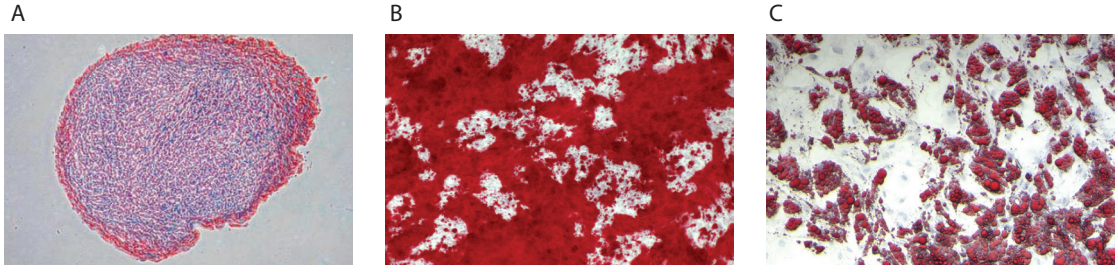


Figure 22. Differentiation potential of human ADSCs. (A) ADSCs induced to differentiate towards chondrocytes for 29 days and then stained with safranin orange (pellet cross-sectional staining) for proteoglycan content. Image captured using 4x objective. **(B)** ADSCs induced to differentiate towards osteoblasts for 29 days and then stained with alizarin red (which stains mineralized extracellular matrix). Image captured using 4x objective. **(C)** ADSCs induced to differentiate towards adipocytes for 14 days and then stained with oil red O (which stains lipid vacuoles) and counterstained with hematoxylin. Image captured using 10x objective.

StemPro® Rat Alk Phos Expressing Mesenchymal Stem Cells

- → Highly characterized for surface antigens
- → Unique ability to track cells in transplantation and differentiation studies
- → Easy detection of alkaline phosphatase activity using the ELF® 97 Endogenous Phosphatase Kit (Figure 23)

StemPro® Rat alkaline phosphatase–expressing MSCs [18] are produced from bone marrow isolated from transgenic Fischer 344 rats expressing the human placental alkaline phosphatase (hPAP) gene linked to the ubiquitously active ROSA26 (R26) gene promoter. Before cryopreservation, the MSCs are expanded for 3 passages in alpha-MEM supplemented with 10% MSC-Qualified FBS and antibiotic/antimycotic solution.

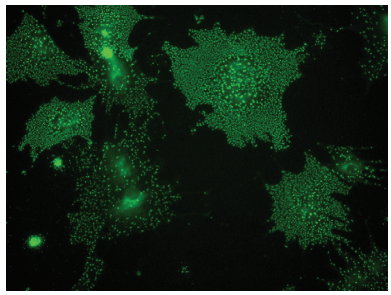


Figure 23. Rat Alk Phos Expressing MSCs imaged using the human placental alkaline phosphatase (hPAP) gene. Rat Alk Phos Expressing MSCs were expanded in alpha-MEM and MSC-qualified FBS, then stained using the ELF® 97 Endogenous Phosphatase Kit.

GIBCO® Mouse (C57BL/6) Mesenchymal Stem Cells

- → Isolated from bone marrow of C57BL/6 mice at ≤8 weeks after gestation
- → Cryopreserved at passage 3 (P3)
- → High post-thaw viability (>70%) (Figure 24)
- → Maintain undifferentiated state with the ability to differentiate into osteocytes, adipocytes, and chondrocytes

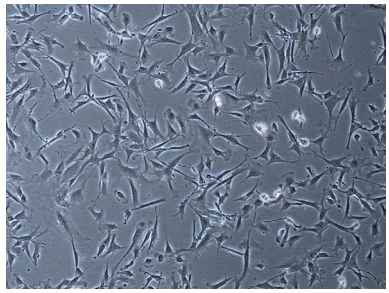


Figure 24. GIBCO® Mouse (C57BL/6) MSCs at passage 1 (P1) post-thaw were expanded for two days in alpha-MEM medium supplemented with 10% MSC-Qualified FBS. The seeding density was 5 x 10^3 cells/cm^2 in a T75 culture vessel.

GIBCO® Rat (Sprague Dawley) Mesenchymal Stem Cells

- → Isolated from bone marrow of Sprague Dawley rats
- → Cryopreserved at passage 3 (P3)
- → High post-thaw viability (>70%) (Figure 25)
- → Maintain undifferentiated state with the ability to differentiate into osteocytes, adipocytes, and chondrocytes

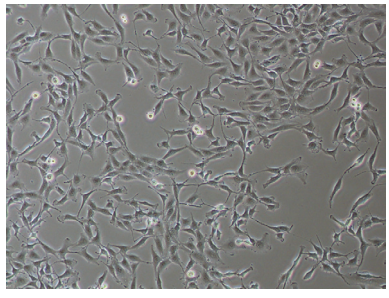


Figure 25. GIBCO® Rat (SD) MSCs thawed and expanded in alpha-MEM medium supplemented with 10% MSC-qualified FBS. The seeding density was 3 x 10^3 cells/cm^2 in a T75 culture vessel.

Ordering information

Product	Quantity	Cat. No.
StemPro® Rat Alk Phos Expressing Mesenchymal Stem Cells	1 mL	R7789-120
GIBCO® Mouse (C57BL/6) Mesenchymal Stem Cells	1 mL	S1502-100
GIBCO® Rat (SD) Mesenchymal Stem Cells	1 mL	S1601-100
Minimum Essential Medium (MEM) alpha Medium (1X), liquid	500 mL	32571-036
MSC-Qualified FBS USDA	100 mL	12662-011
MSC-Qualified FBS USDA	500 mL	12662-029

Learn more at www.invitrogen.com/stemcell/msc.

Neon™ Transfection System

Shockingly simple transfection of stem cells

- → Efficiency—up to 90% in many cell types, including difficult-to-transfect, primary, and stem cells (Figures 26, 27, and 28; Table 4)
- → Flexibility—easily transfect from 2 x 10^4 to 6 x 10^6 cells per reaction
- → Simplicity—single reagent kit for all cell types
- → Versatility—open system allows electroporation parameters to be optimized freely
- → Easy-to-use protocol

Visit www.invitrogen.com/neon for more information.

Table 4. Examples of stem cells successfully transfected with the Neon™ Transfection System.

Cell line	Tissue origin	Transfection efficiency (%)	Viable cells (%)
Mesenchymal stem cells	Bone marrow	54	90
Human adipose-derived stem cells	Lipoaspirate	88	96

Transfection efficiency is calculated from the numbers of live vs. dead cells.

Figure 26. The Neon™ Transfection System offers breakthrough technology for transfection of primary, stem, and other difficult-to-transfect cells.

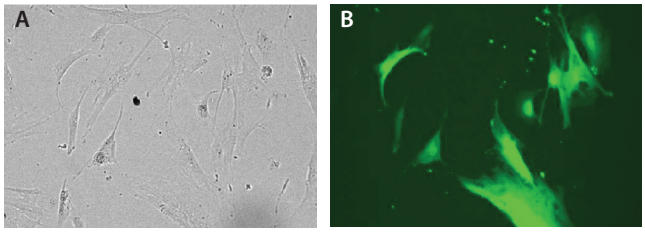


Figure 27. Human MSC cells transfected using the Neon™ Transfection System and 0.5 µg of a plasmid encoding EGFP. At 24 hours posttransfection, the cells were analyzed by **(A)** light and **(B)** fluorescence microscopy.

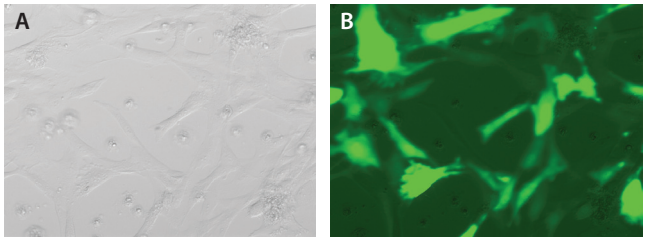


Figure 28. Human adipose-derived stem cells (ADSC) transfected using the Neon™ Transfection System and 0.5 µg of a plasmid encoding EGFP. At 48 hours posttransfection, the cells were analyzed by **(A)** light and **(B)** fluorescence microscopy.

Ordering information

Product	Quantity	Cat. No.
Neon™ Transfection System		
Neon™ Transfection System Starter Pack	1 pack	MPK5000S
Neon™ Transfection System	1 each	MPK5000
Neon™ Transfection System Kits		
Neon™ Transfection System Kit (100 µL)	192 reactions	MPK10096
Neon™ Transfection System Kit (100 µL)	50 reactions	MPK10025
Neon™ Transfection System Kit (10 µL)	192 reactions	MPK1096
Neon™ Transfection System Kit (10 µL)	50 reactions	MPK1025
Neon™ Transfection System accessories		
Neon™ Transfection System Pipette	1 each	MPP100
Neon™ Transfection System Pipette Station	1 each	MPS100
Neon™ Transfection Tubes	1 pack	MPT100
Neon™ Transfection System Extended Warranty	1 each	MPSERV

Stem cell characterization and tracking

Find an extensive portfolio of cellular analysis products at the Cell & Tissue Analysis application storefront (Figure 29, Table 5). Newly revised and continually updated, the site also includes easy access to resources such as our Fluorescence Tutorials and an online version of *The Handbook—A Guide to Fluorescent Probes and Labeling Technologies*. Find detailed information describing the use of more than 3,000 Molecular Probes® products for life science research, including extensive data, numerous technical notes, and full-color images of products in action.

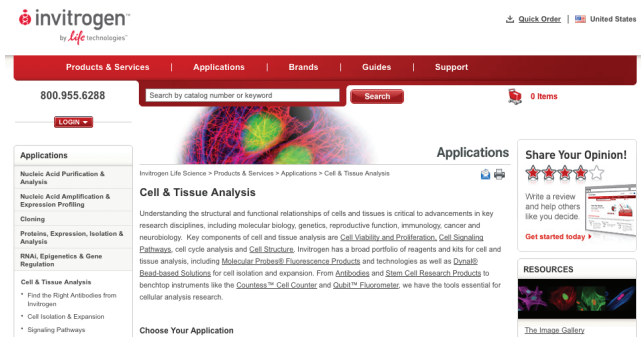


Figure 29. Cell & Tissue Analysis storefront at www.invitrogen.com/cellularanalysis.

Table 5. Online resources. Visit www.invitrogen.com/cellularanalysis and choose your application.

Antibodies and secondary detection	Search for primary antibodies using the LINNEA™ Guide to Antibodies. Easily select secondary antibodies, and find out more about phosphospecific antibodies, products for western blotting, and new antibody introductions.
Cell isolation and expansion	Simplify cell isolation and expansion with Dynabeads® tube-based technology. Also learn more about clinical research cell isolation and expansion.
Signaling pathways	Empower your research using Invitrogen's comprehensive portfolio of products and services to investigate cell signaling pathways, including signal transduction pathways for Akt, MAPK, JAK-STAT, T cell receptors, and more.
Cell structure	Powerful tools to visualize and identify subcellular organelles in live or fixed cells. Find fluorescent protein–based probes ready for delivery to most cell types.
Cell viability, proliferation, and function	A diverse selection of cell health and metabolism assays for the analysis of apoptosis, cell cycle, cell viability, proliferation, endocytosis, and phagocytosis.
Stem cell research	Find the products and information you need for your embryonic, mesenchymal, hematopoietic, and neural stem cell research.
Cell tracing and tracking	Follow changes in cell morphology and movement with Molecular Probes® dyes and stains for neuronal, microbial, and cell tracing.
Cellular imaging	Innovative Molecular Probes® fluorescent reagents and accessories for enhanced fluorescence microscopy, high-content screening, and *in vivo* imaging.
Flow cytometry	Perform your flow cytometry research with Molecular Probes® fluorescence technologies, including novel viability assays and violet laser reagents. A broad range of antibodies for flow cytometry is also offered.
Quantum dots and microspheres	Find our Qdot® nanocrystals and conjugates as well as our vast selection of fluorescent microspheres for imaging, flow cytometry calibration, and *in vivo* imaging.
Immunoassays	For accurate quantitation of intracellular or extracellular proteins, we offer a wide range of reagents to build your own immunoassays, traditional ELISA and phosphoELISA™ kits, and multiplex kits for use with Luminex® technology.
Labeling chemistry	Easily label antibodies, proteins, peptides, ligands, oligos, and other biomolecules with Molecular Probes® Alexa Fluor® dyes and reagents, including APEX™ Antibody Labeling Kits for labeling small amounts of antibodies.
Microbiological analysis	Assay for key metabolic processes and subcellular structures, and monitor growth, proliferation, and vitality in bacteria and yeast.

MSC primary antibodies

We offer a comprehensive library of primary antibodies for mesenchymal stem cells (Figure 30). This library includes antibodies to positive and negative markers identified by the International Society for Cellular Therapy [19] to define human MSCs, leading the way to more easily comparable research results. We also offer custom conjugation of any antibody to the fluorophore of your choice. Each antibody is of high quality and has been extensively tested and validated.

Learn more at www.invitrogen.com/antibodies.

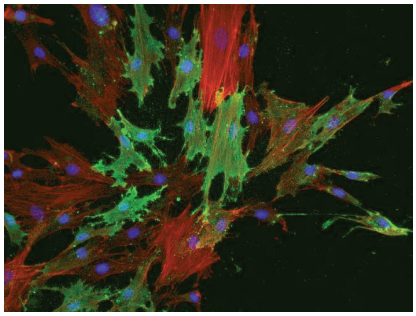


Figure 30. Positive marker for mesenchymal stem cells. Immunofluorescence analysis of mesenchymal stem cells using the mouse anti–Stro-1 (39-8401) and goat anti-mouse Alexa Fluor® 488 (A21042) (green). Actin is stained with phalloidin Alexa Fluor® 594 (red), and nuclei are stained with DAPI (blue). The sample is mounted in ProLong® Gold antifade reagent.

Qtracker® Cell Labeling Kits

- → Excellent tools for long-term studies of MSCs, including migration, motility, morphology, and other cell function assays [21]
- → Several colors are available, for simple, single-excitation multicolor analysis

Qtracker® Cell Labeling Kits deliver fluorescent Qdot® nanocrystals (Figure 31) into the cytoplasm of live cells using a custom targeting peptide. Once inside the cells, Qtracker® labels provide intense, stable fluorescence that can be traced through several generations and are not transferred to adjacent cells in a population.

Learn more at www.invitrogen.com/qdots.

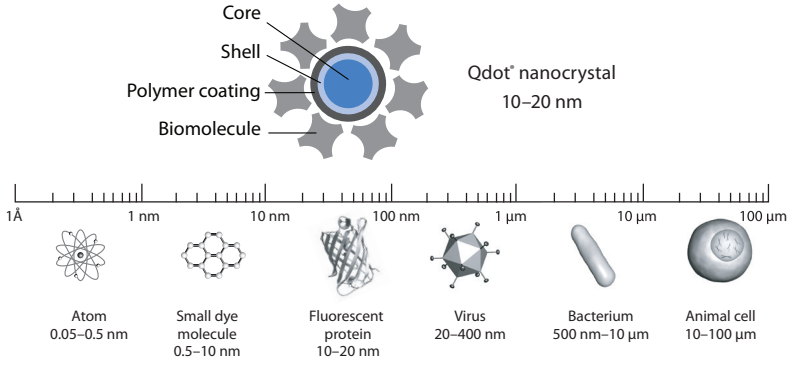


Figure 31. Relative size (hydrodynamic diameter in nm) of Qdot® nanocrystals. Qdot® nanocrystals are roughly protein-sized clusters of semiconductor material.

Ordering information

Product	Quantity	Cat. No.
MSC primary antibodies—positive markers		
CD44	2 mL	MHCD4400-4
CD73	100 µg	41-0200
CD90.2	0.5 mL	MM2005
CD105	0.5 mL	MHCD10500
STRO-1[20]	100 µL	39-8401
MSC primary antibodies—negative markers		
CD11b	0.5 mL	CD11b00
CD14	0.5 mL	MHCD1400
CD19	100 µg	AHS1912
CD34	100 µg	07-3403
CD45	0.5 mL	MHCD4500
CD79a	0.5 mL	MHCD79a00
HLA-DR	1 mL	18-0168
Qtracker® Cell Labeling Kits		
Qtracker® 525	1 kit	Q25041MP
Qtracker® 565	1 kit	Q25031MP
Qtracker® 585	1 kit	Q25011MP
Qtracker® 605	1 kit	Q25001MP
Qtracker® 655	1 kit	Q25021MP
Qtracker® 705	1 kit	Q25061MP
Qtracker® 800	1 kit	Q25071MP

TaqMan® Gene Expression Assays

We offer more than 1 million TaqMan® Gene Expression Assays, a comprehensive set of predesigned real-time PCR assays (Figure 32). All TaqMan® Gene Expression Assays have been designed using our validated bioinformatics pipeline and are run with the same PCR protocol, eliminating the need for primer design or PCR optimization.

Below are the matched gene expression markers for positive and negative protein markers identified by the International Society for Cellular Therapy [19] to define human MSCs, leading the way to more easily comparable expression results.

Gene name	Gene symbol	Assay ID
5'-nucleotidase, ecto (CD73)	NT5E	Hs01573922_m1
Thy-1 cell surface antigen (CD90)	THY1	Hs00174816_m1
Endoglin (CD105)	ENG	Hs00923996_m1
Integrin, alpha M (complement component 3 receptor 3 subunit)(CD11b)	ITGAM	Hs00355885_m1
CD14 molecule	CD14	Hs02621496_s1
CD19 molecule	CD19	Hs99999192_m1
CD34 molecule	CD34	Hs00156373_m1
Protein tyrosine phosphatase, receptor type C (CD45)	PTPRC	Hs00236304_m1
CD79a molecule, immunoglobulin-associated alpha	CD79A	Hs00233566_m1
Major histocompatibility complex, class II, DR alpha	HLA-DRA	Hs00219575_m1

Figure 32. TaqMan® Gene Expression Assays.

TaqMan® Gene Signature Array Plates

These 96-well plates come in Fast and standard formats (Figure 33) and are preconfigured with the most appropriate TaqMan® Gene Expression Assays for a specific biological process, pathway, or disease state.

→ Each plate contains predefined assays and endogenous controls dried down in the wells, ready for accurate assessment of an entire gene signature in one simple experiment.

→ Examples of TaqMan® Gene Signature Array Plates relevant for MSC characterization are listed below:

Ordering information

Product	Quantity	Cat. No.
TaqMan® Array Human Cell Surface Markers Plate	96 reactions	4414109
TaqMan® Array Human BMP Pathway Plate	96 reactions	4414110
TaqMan® Array Human Hypoxia Plate	96 reactions	4414090
TaqMan® Array Human Osteogenesis Plate	96 reactions	4414096

Figure 33. TaqMan® Gene Signature Array Plates.

Visit www.invitrogen.com/taqman for TaqMan® Assay ordering information.

GIBCO® cell culture custom media and services

Media made to your specifications

We realize not all cell culture requirements are alike. We are committed to providing you with GIBCO® products customized to your individual needs—quality media you know and trust, customized to your specifications.

Large-scale cGMP custom media

For large-scale clinical or commercial biomanufacturing applications, rely on world-class validated cGMP custom services (Figure 34).

- → Liquid in batches from 10 L to 10,000 L
- → Dry powder media (DPM) in batches from 1 kg to 8,000 kg
- → Advanced Granulation Technology™ (AGT™) media in batches from 50 kg to 6,000 kg (Figure 35)

Custom packaging options

You have the option of receiving your GIBCO® custom media in the packaging that best suits your needs. We have many different options for liquid and powder media in a variety of package sizes, including small, intermediate, and large scales (Figure 36).

GIBCO® MediaExpress™ and Rapid Research

These services are specifically designed for small-scale, non-cGMP custom orders when speed matters most. We offer GIBCO® product quality in small batches for quick turnaround and smooth transition to cGMP scale-up.

Process development custom services

Choose the PD-Direct® team to reduce process development inefficiencies and improve time and cost performance using our latest technologies.

cGMP manufacturing sites

Life Technologies maintains two primary GIBCO® cell culture manufacturing locations—in the USA and in Scotland—and three primary GIBCO® serum and/or protein product manufacturing locations—in the USA, New Zealand, and Australia. For reliable global service and contingency planning, we welcome visits and audits of our cGMP facilities to help facilitate regulatory approvals of your products and services.

Better cell culture is based on knowledge

Our Account Managers, Technical Support Scientists, R&D Scientists, and Quality Experts are here for you.

Learn more at www.invitrogen.com/bioproduction.

Figure 34. cGMP manufacturing sites.

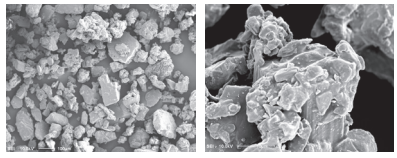


Figure 35. Advanced Granulation Technology™ (AGT™) media.

Figure 36. Custom packaging options.

References

General

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.

StemPro® MSC SFM XenoFree, StemPro® MSC SFM, MesenPRO RS™, and MSC-Qualified FBS

3. Lindroos B et al. (2009) Serum-free, xeno-free culture media maintain the proliferation rate and multipotentiality of adipose stem cells *in vitro*. *Cytotherapy* 11(7):958–972.
4. Sugii S et al. (2010) Human and mouse adipose-derived cells support feeder-independent induction of pluripotent stem cells. *Proc Natl Acad Sci U S A* 107(8):3558–3563.
5. Chase L et al. (2010) A novel serum-free medium for the expansion of human mesenchymal stem cells. *Stem Cell Res Ther* 1:8 doi:10.1186/scrt8.
6. Agata H et al. (2009) Feasibility and efficacy of bone tissue engineering using human bone marrow stromal cells cultivated in serum-free conditions. *Biochem Biophys Res Commun* 382(2):353–358.
7. Ng F et al. (2008) PDGF, TGF-beta, 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 MSCs into adipogenic, chondrogenic, and osteogenic lineages. *Blood* 112(2):295–307.
8. Eibes G et al. (2010) Maximizing the *ex vivo* expansion of human mesenchymal stem cells using a microcarrier-based stirred culture system. *J Biotechnol* 146(4):194–197.
9. Tsigkou O et al. (2010) Engineered vascularized bone grafts. *Proc Natl Acad Sci U S A* 107(8):3311–3316.
10. Schraufstatter IU et al. (2009) C3a and C5a are chemotactic factors for human mesenchymal stem cells, which cause prolonged ERK1/2 phosphorylation. *J Immunol* 182(6):3827–3836.
11. Kuçi S et al. (2010) CD271 antigen defines a subset of multipotent stromal cells with immunosuppressive and lymphohematopoietic engraftment-promoting properties. *Haematologica* 95(4):651–659.
12. Garcia-Gonzalo F, Belmonte J (2008) Albumin-associated lipids regulate human embryonic stem cell self-renewal. *PLoS ONE* 2008; 3(1): e1384. doi: 10.1371/journal.pone.0001384.

Synth-a-Freeze®

13. Sandrine L et al. (2010) Cellular pharmacodynamics of the novel biaryloxazolidinone radezolid: studies with infected phagocytic and non-phagocytic cells, using *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Listeria monocytogenes*, and *Legionella pneumophila*. *Antimicrob Agents Chemother* 10.1128/AAC.01724-09.

StemPro® MSC Differentiation Kits (adipogenesis, chondrogenesis, and osteogenesis)

14. Boucher S et al. (2009) A simplified culture and polymerase chain reaction identification assay for quality control performance testing of stem cell media products. *Cytotherapy* 11(6):761–767.
15. Carro MS et al. (2010) The transcriptional network for mesenchymal transformation of brain tumours. *Nature* Jan 21;463(7279):318–325.
16. Carreras A et al. (2009) Obstructive apneas induce early release of mesenchymal stem cells into circulating blood. *Sleep* 32(1):117–119.

StemPro® Human Adipose-Derived Stem Cells

17. Lee J et al. (2010) Anti-adipogenesis by 6-thioinosine is mediated by down-regulation of PPAR gamma through JNK-dependent upregulation of iNOS. *Cell Mol Life Sci* 67(3):467–481. Epub 2009 Nov 26.

StemPro® Alk Phos Expressing Rat MSC

18. Kisseberth WC et al. (1999) Ubiquitous expression of marker transgenes in mice and rats. *Dev Biol* 214:128–138.
19. Mujtaba T et al. (2002) Stable expression of the alkaline phosphatase marker gene by neural cells in culture and after transplantation into the CNS using cells derived from a transgenic rat. *Exp Neurol* 174:48–57.

MSC characterization and tracking

20. Dominici M et al. (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8:315–17.
21. Simmons PJ, Torok-Storb B (1991) Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO-1. *Blood* 78:55–62.
22. Rosen AB et al. (2007) Finding fluorescent needles in the cardiac haystack: tracking human mesenchymal stem cells labeled with quantum dots for quantitative *in vivo* three-dimensional fluorescence analysis. *Stem Cells* 25:2128–2138.

MSC pathways

23. 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.
24. Caplan AI (2007) Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J Cell Physiol* 3(2):341–347.
25. Phinney DG, Prockop DJ (2007) Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair—current views. *Stem Cells* 25(11):2896–2902.
26. 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.
27. Rosen AB (2007) Finding fluorescent needles in the cardiac haystack: tracking human mesenchymal stem cells labeled with quantum dots for quantitative *in vivo* three-dimensional fluorescence analysis. *Stem Cells* 25(8):2128–38. Epub 2007 May 10.
28. Lindroos B et al. (2009) Serum-free, xeno-free culture media maintain the proliferation rate and multipotentiality of adipose stem cells *in vitro*. *Cytotherapy* 11(7):958–972.
29. Chase L et al. (2010) A novel serum-free medium for the expansion of human mesenchymal stem cells. *Stem Cell Res Ther* 1:8.
30. Ng F et al. (2008) PDGF, TGF-beta, 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 MSCs into adipogenic, chondrogenic, and osteogenic lineages. *Blood* 112(2):295-307.

gibco® | invitrogen™
by life technologies™

www.invitrogen.com

For research use only. Not intended for any animal or human therapeutic or diagnostic use, unless otherwise stated.