

A fluorescence microscopy image showing a network of muscle cells. The cells are stained with three different dyes: blue for nuclei, green for cytoplasm or specific organelles, and red for muscle fibers. The red fibers are arranged in a complex, interconnected pattern, while the green and blue components are more diffuse and punctate.

**gibco**

# MSC resource guide

Key technologies and applications  
for MSC research

**ThermoFisher**  
SCIENTIFIC

# Key technologies for mesenchymal stem cell research

Innovative solutions  
for the entire stem cell research workflow



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

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 from discovery to the clinic.

## Why is manufacturing quality important?

We offer the broadest portfolio of cGMP-manufactured products for MSC research. cGMP compliance helps ensure traceability and manufacturing reliability. Our facility in Grand Island, New York, is a medical device 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. By following these regulatory guidelines and manufacturing according to cGMP we provide high-quality products with lot-to-lot consistency and traceability, helping to ensure the best foundation for reproducible, reliable results.

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 go to [thermofisher.com/stemcells](https://www.thermofisher.com/stemcells)

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# Mesenchymal stem cell 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 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. View the guide in Table 2 for recommendations on developing a complete media system for your MSC research.

**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              | x                      | x                    | x                 | x**             |
| Maintains MSC phenotype                                  | x                      | x                    | x                 | x               |
| Supports growth at high cell density                     |                        | x                    | x                 | x               |
| Supports trilineage differentiation                      | x                      | x                    | x                 | x               |
| Enhanced chondrogenesis                                  |                        | x                    | x                 | x               |
| cGMP manufactured providing reliability and traceability | x                      | x                    | x                 | x               |
| Lot-to-lot consistency                                   |                        | x                    | x                 | x               |
| Designed for cell therapy applications                   |                        |                      | x                 |                 |
| Free of animal components                                |                        |                      |                   | x               |

\* Available with components originating from BSE negligible risk (United States, New Zealand or Australia) countries.

\*\* Requires the supplementation of low-level (i.e., 2%) human AB serum (primary culture only), after which cells can be expanded under completely serum-free/xeno-free conditions.

**Table 2. MSC culture product selection guide.**

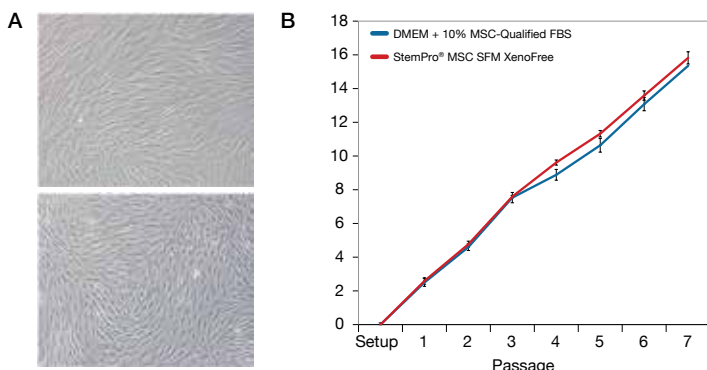
|                      | Serum classical media  | Reduced-serum media                                | Serum-free media                                | Xeno-free media                              |
|----------------------|--|--|---|--|
| Basal media          | DMEM (low glucose) /DMEM (low glucose with GlutaMAX™-I Supplement)             | Gibco™ MesenPRO RS™ Medium                         | Gibco™ CTS™ StemPro™ MSC SFM                    | Gibco™ StemPro™ MSC SFM XenoFree             |
| Supplements          | MSC-Qualified FBS  |  | L-Glutamine, Gibco™ CTS™ GlutaMAX™-I Supplement | L-Glutamine, CTS GlutaMAX-I Supplement       |
| Extracellular matrix |  |  | CTS CELLstart™ Substrate, Fibronectin           | Gibco™ CTS™ CELLstart Substrate, fibronectin |
| Passaging reagents   | TrypLE™ reagents, trypsin, Gibco™ StemPro™ Accutase™ Cell Dissociation Reagent | TrypLE reagents, trypsin, StemPro Accutase reagent | Gibco™ CTS™ TrypLE™ Select Enzyme               | TrypLE reagents                              |
| Species tested       | Human, mouse, rat, dog   | Human, mouse, rat, sheep, goat, pig, horse         | Human   | Human  |

# MSC expansion media

## StemPro MSC SFM XenoFree

- Maintains trilineage mesoderm differentiation potential beyond five passages (Figure 1)
- Maintains MSC surface marker expression (Figure 1) and normal gene expression profiles
- Serum-free and xeno-free medium for MSC expansion, which helps ensure traceability and manufacturing reliability
- Complete xeno-free system with CTS CELLstart Substrate to enable MSC attachment under serum-free conditions

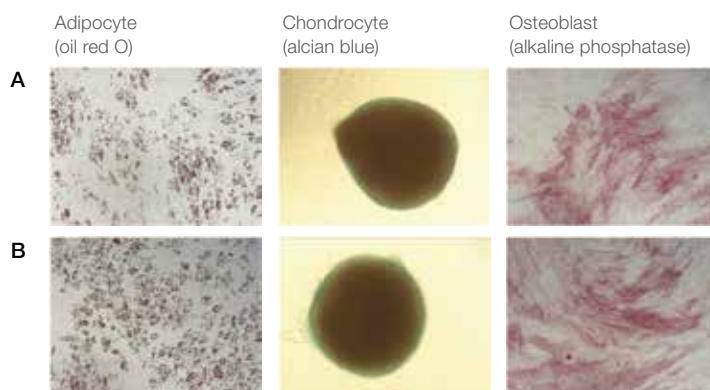
Due to the low frequency of human MSCs in primary tissue, expansion of this stem cell population is critical and helps enable basic biological studies and clinical research. In addition, human MSCs can only be propagated a limited number of times, thereafter exhibiting reduced proliferation and differentiation 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).



**Figure 2. Human MSC expansion under xeno-free conditions.** Human bone marrow–derived MSCs expanded in DMEM (low glucose) + 10% MSC-Qualified FBS or 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 =  $5\text{--}7 \times 10^3$  cells/cm<sup>2</sup>. Harvest enzyme = TrypLE™ Express Enzyme. Counting method = Countess™ Automated Cell Counter.

StemPro MSC SFM XenoFree offers a xeno-free system at the primary component level when used in conjunction with CTS CELLstart Substrate; thus, cells are grown in a more physiologically relevant environment that allows for more clinically relevant results.

Get more information at [thermofisher.com/mscxenofree](https://thermofisher.com/mscxenofree)



**C**

| DMEM + 10% MSC-Qualified FBS         |            |
|--------------------------------------|------------|
| Marker                               | % Positive |
| CD73 <sup>+</sup> /NEG <sup>-</sup>  | 99.7       |
| CD90 <sup>+</sup> /NEG <sup>-</sup>  | 99.6       |
| CD105 <sup>+</sup> /NEG <sup>-</sup> | 100.0      |
| CD34 <sup>+</sup>                    | 0.4        |

| StemPro MSC SFM XenoFree             |            |
|--------------------------------------|------------|
| Marker                               | % Positive |
| CD73 <sup>+</sup> /NEG <sup>-</sup>  | 98.2       |
| CD90 <sup>+</sup> /NEG <sup>-</sup>  | 99.6       |
| CD105 <sup>+</sup> /NEG <sup>-</sup> | 100.0      |
| CD34 <sup>+</sup>                    | 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.

# 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]

## Media ordering information

| Product                   | Quantity | Cat. No. |
|---------------------------|----------|----------|
| CTS CELLstart Substrate*  | 2 mL     | A1014201 |
| Coating Matrix Kit        | 1 kit    | R011K    |
| Fibronectin, human plasma | 5 mg     | 33016015 |
| GlutaMAX-I Supplement     | 100 mL   | 35050061 |
| L-Glutamine               | 20 mL    | 25030149 |
| StemPro MSC SFM XenoFree  | 1 kit    | A1067501 |
| CTS TrypLE Select Enzyme* | 100 mL   | A1285901 |

\*For Research Use or Manufacturing of Cell, Gene, or Tissue-Based Products. CAUTION: Not intended for direct administration into humans or animals.

### Passaging

- TrypLE reagent

### Cells

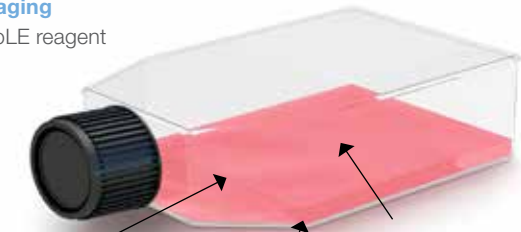
Species: Human  
Origin: Bone marrow  
Adipose  
Cord blood  
Pericytes  
Fibroblasts

### Cell culture media

- StemPro MSC SFM XenoFree
- L-Glutamine/  
GlutaMAX-1 Supplement

### Extracellular matrices

- Fibronectin
- CELLstart Substrate

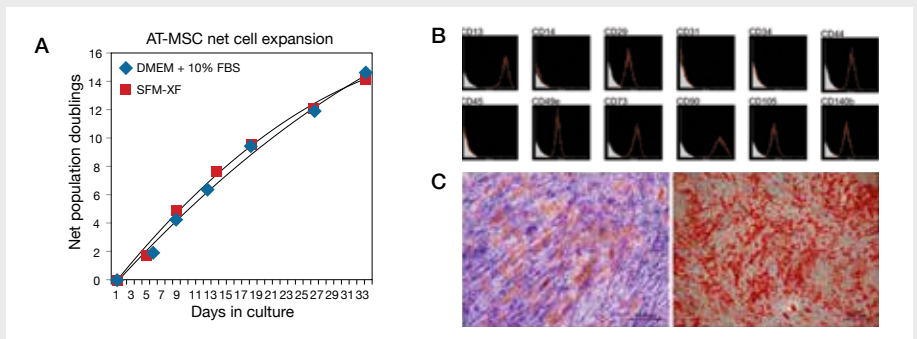


## Customer testimonial

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, São Paulo, Brazil

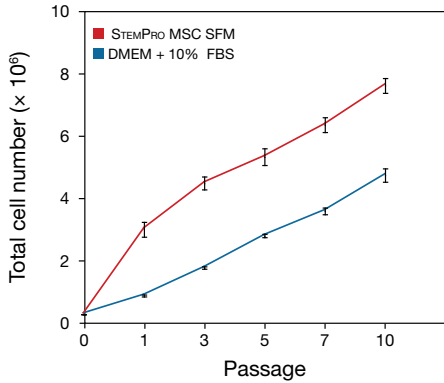


**Figure 3. Expansion, differentiation, and characterization of human AT-MSCs grown under xeno-free conditions.** (A) Human AT-MSCs expanded in classical (10% FBS-containing) medium or StemPro MSC SFM XenoFree on CELLstart Substrate-coated plates revealed similar levels of cumulative cell growth (cumulative 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 a 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.

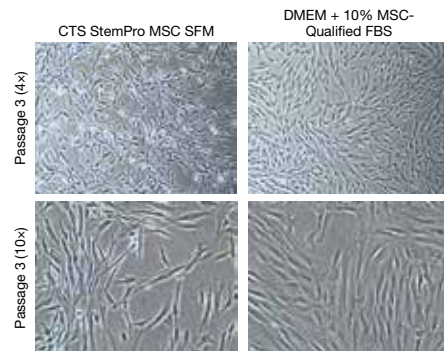
# CTS StemPro MSC Serum-Free Medium (SFM)

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

- Improved expansion when 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 five passages (Figure 6)



**Figure 4.** hMSCs grown on CELLstart Substrate-coated dishes in CTS 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 CTS StemPro MSC SFM and classical medium (n = 3). The culture had a seed density of 1 × 10<sup>4</sup> cells/cm<sup>2</sup>, a split frequency of 3 days, and a medium change every 2 days.



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

- Batch-to-batch consistency, 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 (United States, 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

CTS StemPro MSC SFM [5–7] 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 between 1 × 10<sup>4</sup> and 3 × 10<sup>4</sup> cells/cm<sup>2</sup>, human MSCs grown in CTS StemPro MSC SFM have a much smaller, spindle-shaped morphology and can reach densities >1 × 10<sup>5</sup> cells/cm<sup>2</sup> (Figure 5).

Learn more at

[thermofisher.com/stempro/msc](https://thermofisher.com/stempro/msc)

**Table 3. Benefits of CTS StemPro MSC SFM compared to classical media: better quality, more cells, less cost.**

|                                      | Classical media       | CTS 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 × 10 <sup>6</sup> | 1.6 × 10 <sup>7</sup> |

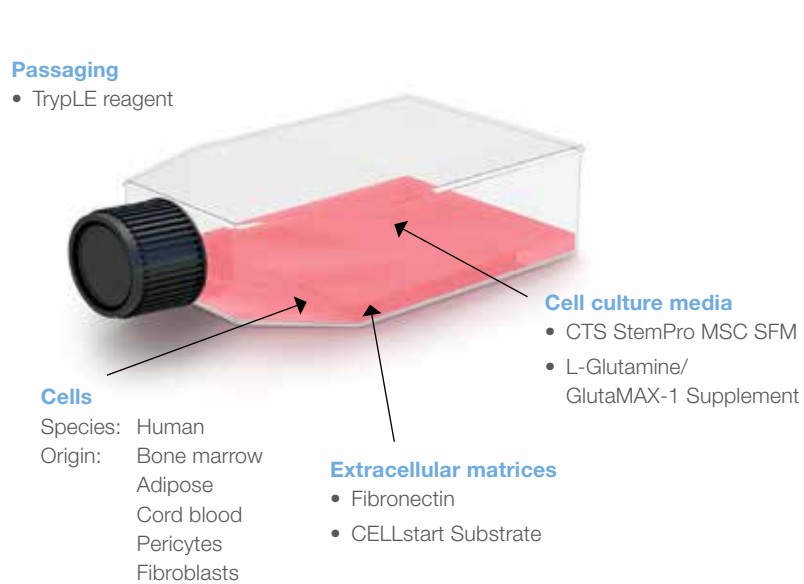


**Figure 6.** hMSCs cultured in CTS StemPro MSC SFM retain trilineage differentiation potential through long-term passaging. hMSCs cultured in CTS StemPro MSC SFM (after passage 5) were seeded into adipogenic, chondrogenic, or osteogenic differentiation medium for 14 days, revealing adipocytes (oil red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



# CTS StemPro MSC SFM Culture System applications

- Derivation, growth, and expansion including high density culture
- Generation of mesoderm lineages
- Optimized for cell therapy applications
- For human *ex vivo* tissue and cell culture processing applications. CAUTION: When used as a medical device, Federal Law restricts this device to sale by or on the order of a physician



## Media system ordering information

| Product                   | Quantity | Cat. No. |
|---------------------------|----------|----------|
| Attachment Factor Protein | 100 mL   | S006100  |
| CTS CELLstart Substrate   | 2 mL     | A1014201 |
| Fibronectin, human plasma | 5 mg     | 33016015 |
| GlutaMAX-I Supplement*    | 100 mL   | 35050061 |
| L-Glutamine               | 20 mL    | 25030149 |
| StemPro MSC SFM           | 1 kit    | A1033201 |
| TrypLE Select Enzyme*     | 100 mL   | 12563011 |

\* Also available as a Cell Therapy Systems™ (CTS™) product.

# 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 that reduces the variability introduced by adding 10% to 20% FBS (typically used in classical media)
- Reduces the time and money spent prequalifying FBS lots
- Batch-to-batch consistency, traceability, and manufacturing reliability

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

Visit [thermofisher.com/mesenpro](http://thermofisher.com/mesenpro) to learn more.

## MesenPRO RS culture system applications

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

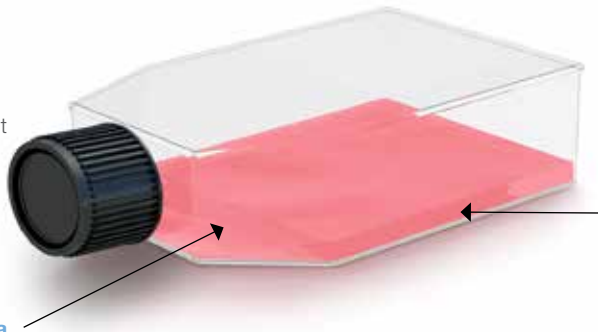
## MSC-Qualified Fetal Bovine Serum (FBS)

### Passaging

- TrypLE reagent
- Trypsin
- StemPro Accutase reagent

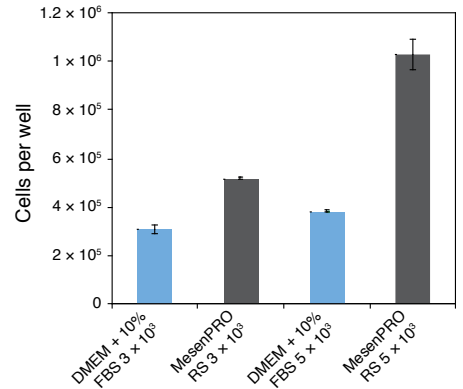
### Cell culture media

- MesenPRO RS Medium
- L-Glutamine/GlutaMAX-I Supplement



### Cells

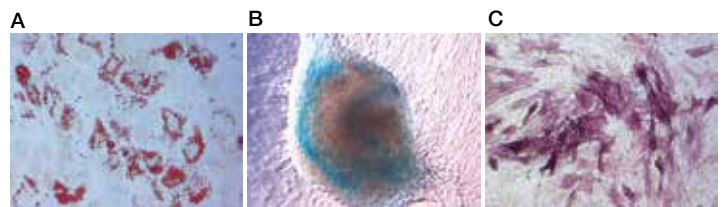
Species: Human  
 Mouse  
 Sheep  
 Pig  
 Origin: Bone marrow  
 Adipose  
 Umbilical cord



**Figure 7. MesenPRO RS Medium provides a 69–169% improvement in expansion over six days compared to classical medium.** MSCs were isolated from normal human bone marrow mononuclear cells by standard techniques. Early-passage cells were plated into 6-well plates at either  $3 \times 10^3$  or  $5 \times 10^3$  cells per  $\text{cm}^2$  in DMEM (low glucose) containing 4 mM L-glutamine, 5  $\mu\text{g}/\text{mL}$  gentamicin, and 10% MSC-Qualified FBS; or MesenPRO RS Medium containing 2 mM L-glutamine and 5  $\mu\text{g}/\text{mL}$  gentamicin. Cells were incubated at  $37^\circ\text{C}$  with 5%  $\text{CO}_2$  in humidified air and fed on day 3. On day 6, cells were harvested using TrypLE reagent and counted with an automated cell counter. Data represent cell count averages from duplicate wells ( $P \leq 0.007$  and  $P \leq 0.002$ , respectively, by Student's *t*-test).

## Media system ordering information

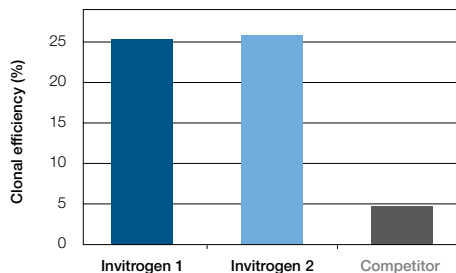
| Product                                 | Quantity | Cat. No. |
|---|----------|----------|
| GlutaMAX-I Supplement                   | 100 mL   | 35050061 |
| L-Glutamine                             | 20 mL    | 25030149 |
| MesenPRO RS Medium                      | 1 kit    | 12746012 |
| MesenPRO RS Medium (New Zealand origin) | 1 kit    | E071000  |
| TrypLE Select Enzyme                    | 100 mL   | 12563011 |
| StemPro Accutase reagent                | 100 mL   | A1110501 |
| Trypsin                                 | 100 mL   | 25300054 |



**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).

- 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 concentration 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 pretesting 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.



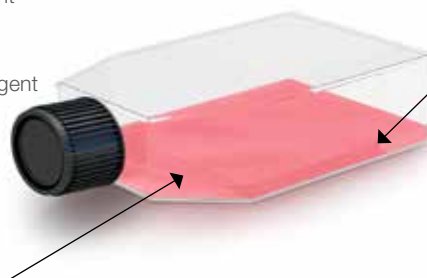
**Figure 9. Effect of FBS source on MSC clonal efficiency.** Mesenchymal stem cells were isolated from normal human bone marrow mononuclear cells by standard techniques. Early-passaged cells were plated into duplicate 100 mm tissue culture dishes at a seeding density of 100 cells per plate in DMEM (low 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. Our MSC-Qualified FBS outperformed a competitor’s MSC-Qualified FBS ( $P < 0.05$ ; Student’s  $t$ -test).

Find out more at [thermofisher.com/stemcell/msc](http://thermofisher.com/stemcell/msc)

## MSC cryopreservation

### Passaging

- TrypLE reagent
- Trypsin
- StemPro Accutase reagent

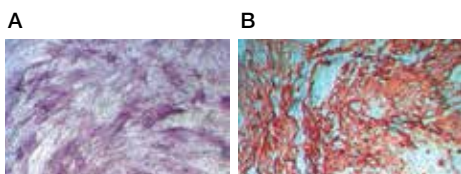


### Cell culture media

- DMEM (low glucose)/MEMα
- MSC-Qualified FBS (10%)
- L-Glutamine/GlutaMAX-I Supplement

### Cells

- Species: Human  
 Mouse  
 Rat  
 Canine  
 Pig  
 Horse
- Origin: Bone marrow  
 Adipose  
 Cord blood  
 Placenta  
 Umbilical cord



**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  $5 \times 10^3$  cells per  $\text{cm}^2$  in 12-well plates. Two hours after seeding, the medium was changed and supplemented with 100 nM dexamethasone, 10 mM sodium  $\beta$ -glycerophosphate, 50 µM ascorbic acid-2-phosphate, and 10 ng/mL BMP-2. Plates were fed every three to four 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.

### MSC-Qualified FBS ordering information

| Product                                    | Quantity | Cat. No. |
|--|----------|----------|
| Minimum Essential Medium (MEM)α*           | 500 mL   | 32571036 |
| DMEM (low glucose)                         | 500 mL   | 11054020 |
| GlutaMAX-I Supplement                      | 100 mL   | 35050061 |
| L-Glutamine                                | 20 mL    | 25030149 |
| MSC-Qualified FBS, USDA*                   | 100 mL   | 12662011 |
| MSC-Qualified FBS, USDA*                   | 500 mL   | 12662029 |
| MSC-Qualified FBS, USDA*                   | 50 mL    | 12662002 |
| MSC-Qualified FBS, Australia*              | 500 mL   | 12664025 |
| MSC-Qualified FBS, New Zealand*            | 100 mL   | 12665014 |
| MSC-Qualified FBS, New Zealand*            | 500 mL   | 12665022 |
| StemPro Accutase Cell Dissociation Reagent | 100 mL   | A1110501 |
| TrypLE Select Enzyme                       | 100 mL   | 12563011 |
| Trypsin-EDTA                               | 100 mL   | 25300054 |

\*For *In Vitro* Diagnostic Use.

# Synth-a-Freeze Medium

## Defined, protein-free cryopreservation medium

- Convenient 1X format cryopreservation system that can be used with any standard freezing protocol
- Gibco™ Synth-a-Freeze™ Cryopreservation Medium performs as well as standard, serum-containing cryopreservation medium for a variety of stem cell types and primary cell lines, including MSCs (Figure 11), ESCs, iPSCs, keratinocytes, fibroblasts, epithelial, and endothelial cells [13]

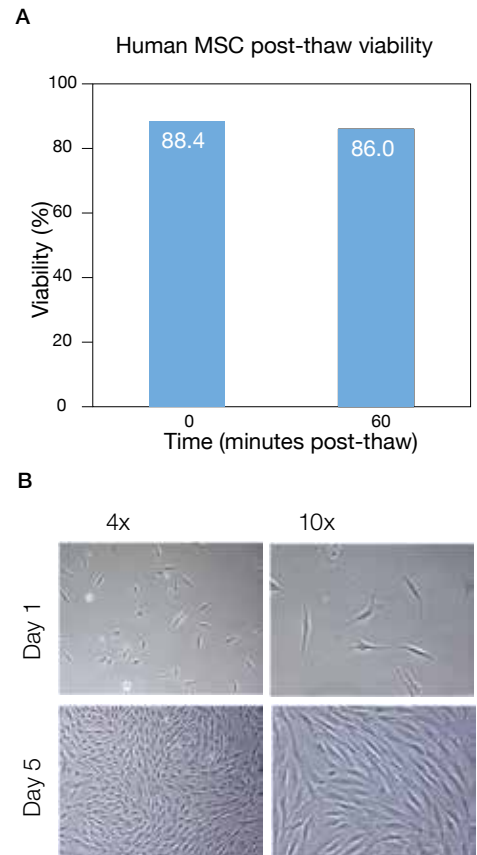
Synth-a-Freeze medium is sterile-filtered, defined, liquid cryopreservation medium containing 10% dimethylsulfoxide (DMSO). Synth-a-Freeze medium does not contain antibiotics, antimycotics, hormones, growth factors, serum, or proteins, and offers an easy-to-use, convenient cryopreservation system.

# Extracellular matrices

### Ordering information

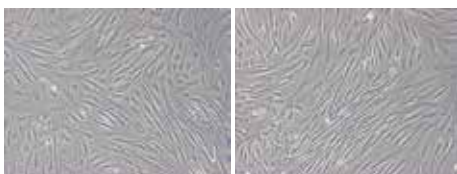
| Product                                 | Quantity | Cat. No. |
|---|----------|----------|
| Synth-a-Freeze Cryopreservation Medium* | 50 mL    | A1254201 |

\* Also available as a Cell Therapy Systems (CTS) product.



**Figure 11. 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 medium and expanded in DMEM + 10% MSC-Qualified FBS for 5 days.

# (ECM) and MSC passaging

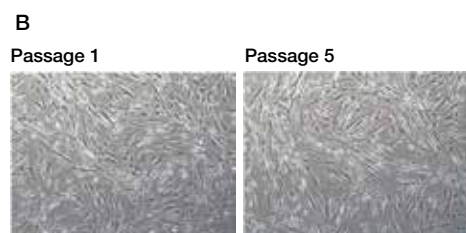


**Figure 12. Multi-passage human MSC expansion on CTS CELLstart Substrate.**

Human MSCs from a 4-donor, passage 4 pool were expanded for 9 passages in StemPro MSC SFM XenoFree on CTS CELLstart Substrate-coated flasks and exhibit a less flattened spindle-shaped morphology.

**A**

| Target                            | % Relative expression (TrypLE reagent vs. trypsin) | % Positive |
|-----------------------------------|--|------------|
| <b>Positive human MSC markers</b> |  |            |
| CD90                              | 106.71   | 98.89      |



**Figure 13. 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 show expression of positive marker CD90. “% Relative expression” is a comparison between human MSCs treated with TrypLE reagent or trypsin. **(B)** Morphology of human MSCs expanded (Passage 1 and 5) in CTS StemPro MSC SFM on CTS CELLstart Substrate-coated plates and dissociated with TrypLE reagent.

## CTS CELLstart Substrate

### First xeno-free, fully defined cell culture substrate

- Maintains multipotency, morphology, and trilineage mesoderm differentiation potential of human MSCs (Figure 12)
- Consistent lot-to-lot performance
- Traceability and manufacturing reliability

CTS CELLstart Substrate is the first xeno-free substrate that contains components only of human origin. CTS 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].

Find out more at [thermofisher.com/3D-cellculture](http://thermofisher.com/3D-cellculture)

## TrypLE Select reagent

### 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

TrypLE Select Enzyme maintains normal morphology of MSC and surface marker expression (Figure 13). 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.

Find out more at [thermofisher.com/trypleselect](http://thermofisher.com/trypleselect)

### Ordering information

| Product                   | Quantity | Cat. No. |
|---------------------------|----------|----------|
| CTS CELLstart Substrate*  | 2 mL     | A1014201 |
| Fibronectin, human plasma | 5 mg     | 33016015 |
| Attachment Factor Protein | 100 mL   | S006100  |
| StemPro Accutase          | 100 mL   | A1110501 |
| Trypsin-EDTA              | 100 mL   | 25300054 |
| TrypLE Select Enzyme      | 100 mL   | 12563011 |

\*For Research Use or Manufacturing of Cell, Gene, or Tissue-Based Products. CAUTION: Not intended for direct administration into humans or animals.

# MSC research overview

## Mesenchymal stem cell research

### Isolation

#### Bone marrow

- Gibco™ 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

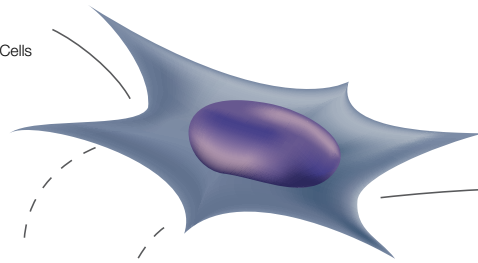
#### Amniotic fluid

#### 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:

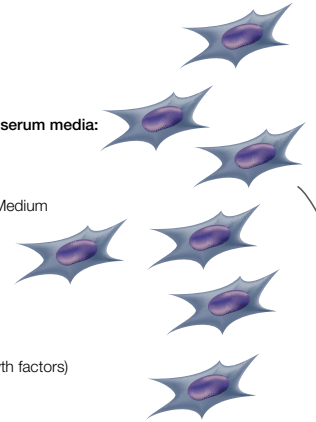
- Complete medium
- StemPro™ MSC SFM XenoFree
- StemPro™ MSC SFM (serum-free)
- MesenPRO RS (reduced-serum: 2%) Medium

#### Classical human MSC media:

- Basal medium
- DMEM (low glucose)
- MEMa

#### Supplement:

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



### Differentiation

Gibco™ StemPro™ Osteogenesis Differentiation Kit

Gibco™ StemPro™ Chondrogenesis Differentiation Kit

5-Azacytidine  
Amphotericin B



Osteoblast



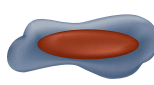
Bone

#### Markers:

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

#### Staining:

- Alkaline phosphatase
- Alizarin red S
- von Kossa



Chondrocyte



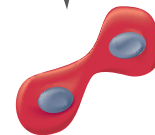
Cartilage

#### Markers:

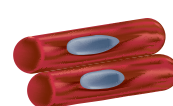
- Aggrecan 1 (AGC1)
- Biglycan (BGN)
- Decorin (DCN)
- Annexin VI (ANXA6)
- Matriin 1 (MATN1)
- Matrix metalloproteinase 13 (MMP13)
- Sox9
- Collagen, type II, alpha 1 (COL2A1)
- Cartilage oligomeric matrix protein (COMP)

#### Staining:

- Alcian blue
- Toluidine blue



Myoblast fusion



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)

# Characterization

## Alternative human MSC media:

Basal medium  
 DMEM (high glucose)  
 DMEM/F-12  
 DDM (low-glucose)/MCD 201  
 IMDM  
 RPMI 1640

## Supplement

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

## Chemokine receptors:

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

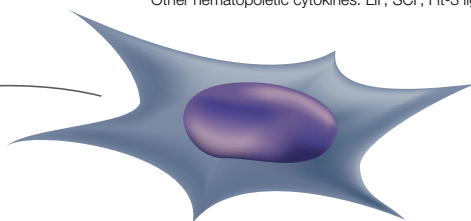
## Cytokine production:

Interleukins: 1 $\alpha$ , 1 $\beta$ , 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, CD79a, 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

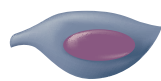
## Clinical trial applications:

Cell replacement therapy  
 Trophic support  
 Antiapoptotic applications  
 Immune modulation

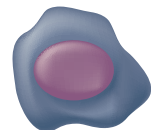
Gibco™ StemPro™ Adipogenesis  
 Differentiation Kit

IL-1 $\alpha$

GDF5, 6, 7  
 Mechanical loading



Stromal fibroblast



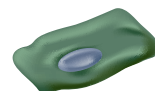
Stroma

## Markers:

Melanoma adhesion molecule (MCAM)  
 Fibroblast activation protein alpha (FAP)  
 Multimerin 2 (MMPN2)  
 Tumor endothelial marker 1 (CD248)



Tenoblast



Tendon

## Markers:

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



Preadipocyte



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 and growth factors

## MSC differentiation kits

### Standardized protocols for human MSC differentiation

- Reliable induction of human MSCs into osteoblasts (Figure 14), chondrocytes (Figure 15), and adipocytes (Figure 16)
- Complements StemPro MSC SFM XenoFree, CTS StemPro MSC SFM, MesenPRO RS Medium, and MSC-Qualified FBS-containing cell expansion systems
- Each lot performance-qualified using PCR
- Reconstituted differentiation kits (basal medium plus supplement) are stable for up to one month
- Supports differentiation of human, mouse, and rat MSCs

Human MSCs differentiate to adipocytes, chondrocytes, and osteoblasts under the 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,

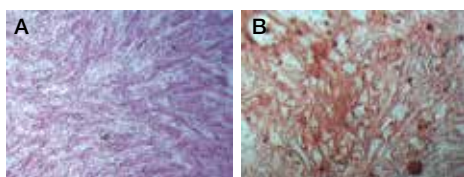
researchers report variable success in differentiation efficiencies arising from quality differences in the raw materials used to generate differentiation cocktails. This issue is further compounded by the differentiation cocktails' serum requirement, which is a major source of lot-to-lot inconsistency. These kits provide researchers with all the necessary pre-qualified components to help reduce that variability.

Find out more at [thermofisher.com/stempro/mscdiff](https://thermofisher.com/stempro/mscdiff)

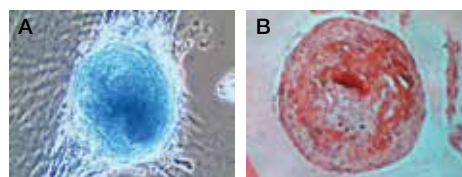
## Growth factors

### Differentiation kit ordering information

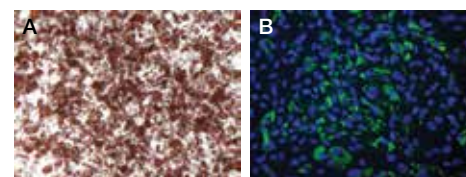
| Product                                    | Quantity | Cat. No. |
|--|----------|----------|
| StemPro Adipogenesis Differentiation Kit   | 1 kit    | A1007001 |
| StemPro Chondrogenesis Differentiation Kit | 1 kit    | A1007101 |
| StemPro Osteogenesis Differentiation Kit   | 1 kit    | A1007201 |



**Figure 14. 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 for 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 15. 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 day 20 chondrogenic pellet.



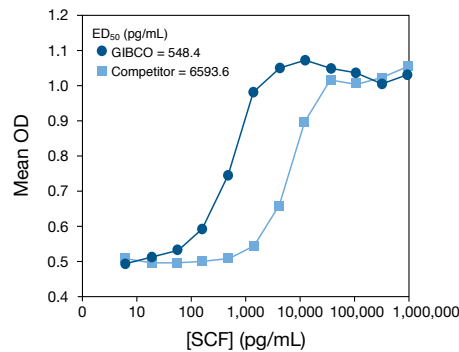
**Figure 16. Adipogenesis-induced differentiation of bone marrow-derived hMSCs using the StemPro Adipogenesis Differentiation Kit. (A)** hMSCs were induced under adipogenic conditions for 14 days, fixed, and stained for oil red O, a marker for lipid-rich vesicles. **(B)** hMSCs were induced under adipogenic conditions for 7 days, fixed, and lipid vesicles visualized with Invitrogen™ Molecular Probes™ HCS LipidTOX™ Green Neutral Lipid Stain (green). Hoechst 33342 was applied as a nuclear counterstain (blue).



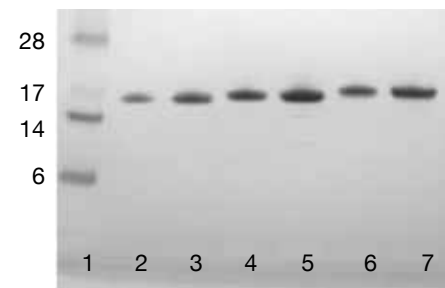
## Gibco growth factors provide the activity, stability, and validation required for your stem cell research

- High biological activity—more results with less protein (Figure 17)
- High purity—no interference from other proteins or contaminants (Figure 18)
- Proved compatibility—Gibco proteins are bioassayed with Gibco media
- Convenient access—Gibco proteins can be stocked in your Invitrogen Supply Center

Learn more at [thermofisher.com/growthfactors](http://thermofisher.com/growthfactors)



**Figure 17. More bioactive protein due to exceptional refolding techniques.** Proliferation of MO7e cells in response to Gibco™ SCF (Cat. No. PHC2115) and the competitor's SCF. As illustrated by the lower ED<sub>50</sub>, less Gibco SCF is required to yield a response.



**Figure 18. 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 (AA 10–155); lane 5: 2 µg FGF-basic (AA 10–155); lane 6: 1 µg FGF basic (FL); lane 7: 2 µg FGF-basic (FL).

## Ordering information

| Product                             | Quantity                         | Cat. No.                                 |
|-------------------------------------|----------------------------------|--|
| BMP-2 (human)                       | 10 µg                            | PHC7145                                  |
| BMP-4 (human)                       | 5 µg                             | PHC7914                                  |
| BMP-7 (active) (human)              | 10 µg<br>10 µg                   | PHC7204<br>PHC7104                       |
| CTGF (human)                        | 20 µg                            | PHG0286                                  |
| EGF (human)                         | 25 µg                            | PHG0315                                  |
| *FGF-basic (full length) (human)    | 10 µg<br>25 µg<br>100 µg<br>1 mg | PHG0264<br>PHG0266<br>PHG0261<br>PHG0263 |
| FGF-basic (human)                   | 10 µg                            | 13256029                                 |
| FGF-basic, (AA 10-155) (human)      | 10 µg<br>50 µg<br>100 µg<br>1 mg | PHG0024<br>PHG0026<br>PHG0021<br>PHG0023 |
| Insulin (human)                     | 5 mL                             | 12585014                                 |
|                                     | 2 µg<br>5 µg                     | PHC0014<br>PHC0015                       |
| IL-1α (human)                       | 25 µg<br>100 µg<br>1 mg          | PHC0017<br>PHC0011<br>PHC0013            |
| Myelin Basic Protein (MBP) (bovine) | 10 mg                            | 13228010                                 |
|                                     | 5 µg<br>10 µg                    | PHG0044<br>PHG0045                       |
| PDGF (human)                        | 50 µg<br>100 µg<br>1 mg          | PHG0046<br>PHG0041<br>PHG0043            |
| TGF-α (human)                       | 100 µg                           | PHG0051                                  |
|                                     | 5 µg<br>10 µg                    | PHG9204<br>PHG9214                       |
| TGF-β1* (human)                     | 100 µg<br>250 µg<br>1 mg         | PHG9211<br>PHG9202<br>PHG9203            |
| TGF-β2 (human)                      | 5 µg                             | PHG9114                                  |
| TGF-β3 (human)                      | 5 µg<br>250 µg                   | PHG9305<br>PHG9302                       |

\*Also available as a Cell Therapy Systems (CTS) product.

## Gibco quality assurance

To help ensure Gibco recombinant proteins are of highest quality, each protein is analyzed for purity along with refolding and structural homogeneity to produce a biologically active protein, in-house bioactivity testing includes cell proliferation, cytotoxicity, calcium flux, secondary cytokine up-regulation, induction of surface antigen expression, and protease assays.

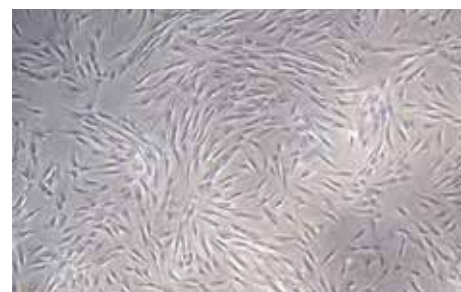
# MSCs and engineered MSCs

## StemPro Human Adipose–Derived Stem Cells (ADSCs)

- Gibco™ StemPro™ Human Adipose–Derived Stem Cells are passaged only once after isolation from human lipoaspirate tissue before cryopreservation (Figure 19)
- After thawing and expansion, StemPro 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 (Table 4)
- Expanded in MesenPRO RS Medium—a reduced-serum (2%) medium that reduces ADSC doubling times (Figure 19)
- Each lot of StemPro 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 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 an equal potential to differentiate into cells and tissues of mesodermal origin, such as adipocytes, cartilage and bone (Figure 20).

Find out more at [thermofisher.com/stempro/adsc](http://thermofisher.com/stempro/adsc)



**Figure 19. Human ADSCs in culture at passage 3.** Phase-contrast image of human ADSCs expanded in MesenPRO RS Medium.

### Ordering information

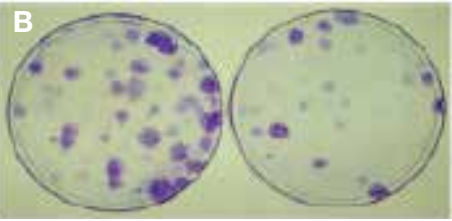
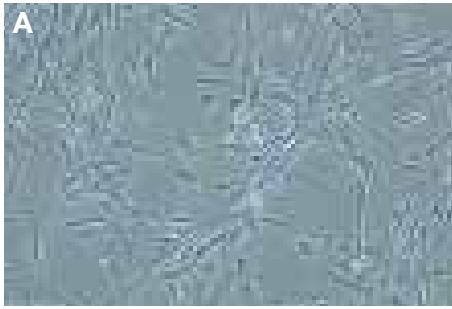
| Product                                      | Quantity | Cat. No. |
|--|----------|----------|
| StemPro™ Human Adipose–Derived Stem Cells    | 1 vial   | R7788115 |
| StemPro™ Human Adipose–Derived Stem Cell Kit | 1 kit    | R7788110 |



**Figure 20. Differentiation potential of human ADSCs.** (A) ADSCs induced to differentiate toward chondrocytes for 29 days and then stained with safranin O (pellet cross-sectional staining) for proteoglycan content; image captured using 4x objective. (B) ADSCs induced to differentiate toward osteoblasts for 29 days and then stained with alizarin red S (which stains mineralized extracellular matrix); image captured using 4x objective. (C) ADSCs induced to differentiate toward adipocytes for 14 days and then stained with oil red O (which stains lipid vacuoles) and counterstained with hematoxylin; image captured using 10x objective.

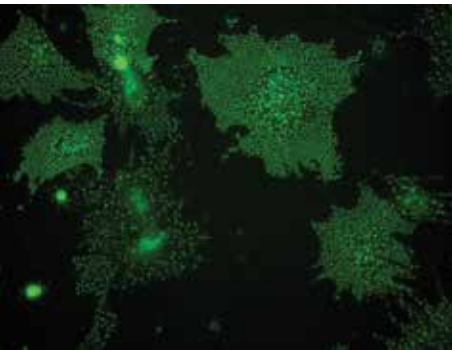
| Marker | >95% positive events | <2% positive events |
|--------|----------------------|---------------------|
| CD14   |                      | X                   |
| CD29   | X                    |                     |
| CD31   |                      | X                   |
| CD44   | X        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

**Table 4. 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.



**Figure 21. StemPro BM MSC morphology and colony formation under hypoxic and normal conditions.**

(A) MSCs retain their fibroblast spindle-like morphology in StemPro MSC XenoFree media. (B) 100 MSCs per dish were plated and grown for 12 days either under hypoxic (left dish) or normoxic (right dish) conditions without changing media. A CFU-F assay was then performed. More colonies were observed after culturing under hypoxic conditions.



**Figure 22. Rat Alk Phos-expressing MSCs.** Fluorescence image of Rat Alk Phos-expressing MSCs expanded in MEM $\alpha$  and MSC-Qualified FBS, stained using ELF 97 Endogenous Phosphatase Kit.

## StemPro BM Mesenchymal Stem Cells

- Minimized assay variability
- Improved functionality studies with immature, high potency cells
- No observed tumorigenicity or toxicity in GLP-compliant animal studies
- Full documentation citing quality control specifications, test results, and donor information

New to our offering of somatic and progenitor cells, Gibco™ StemPro™ BM Mesenchymal Stem Cells are off-the-shelf bone marrow-derived mesenchymal stem cells. These cells are manufactured in compliance with GMP manufacturing standards in a California-licensed facility and are available for nonhuman Research Use Only. Unique to these cells is the low-oxygen manufacturing process in which they are isolated and expanded. Cells manufactured in reduced oxygen environments result in higher yields of potent immature stem cells compared to cells expanded in normal conditions (Figure 21).

## 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 Invitrogen™ Molecular Probes™ ELF™ 97 Endogenous Phosphatase Kit (Figure 22)

Gibco™ StemPro™ Rat Alk Phos-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 three passages in MEM $\alpha$  supplemented with 10% MSC-Qualified FBS and antibiotic/antimycotic solution.

### Ordering information

| Product  | Quantity                       | Cat. No. |
|--|--------------------------------|----------|
| StemPro BM Mesenchymal Stem Cells                      | 1 x 10 <sup>6</sup> cells/vial | A15652   |
| StemPro BM Mesenchymal Stem Cells                      | 5 x 10 <sup>6</sup> cells/vial | A15653   |
| StemPro Rat Alk Phos-expressing Mesenchymal Stem Cells | 1 mL                           | R7789120 |
| Minimum Essential Medium (MEM) $\alpha$ *              | 500 mL                         | 32571036 |
| MSC-Qualified FBS, USDA*                               | 100 mL                         | 12662011 |
| MSC-Qualified FBS, USDA*                               | 500 mL                         | 12662029 |

\* For *In Vitro* Diagnostic Use

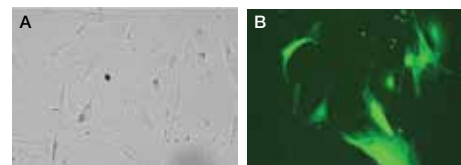
# MSC transfection

## Neon Transfection System

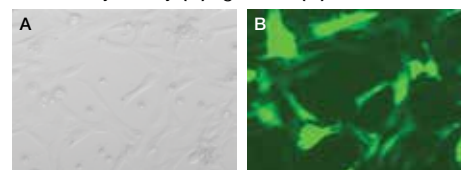
### Surprisingly simple transfection in stem cells

- Efficiency—up to 90% in many cell types, including difficult-to-transfect cells, primary, and stem cells (Figures 23 and 24; Table 4)
- Flexibility—easily transfect from  $2 \times 10^4$  cells to  $6 \times 10^6$  cells per reaction
- Simplicity—single reagent kit for all cell types
- Versatility—open system allows electroporation parameters to be freely optimized
- Easy-to-use protocol

Visit [thermofisher.com/neon](http://thermofisher.com/neon) for more information.



**Figure 23.** Human MSC cells were transfected using the Neon transfection system and 0.5  $\mu\text{g}$  of a plasmid encoding the enhanced green fluorescent protein (EGFP). 24 hours posttransfection, the cells were analyzed by (A) light and (B) fluorescence



**Figure 24.** Human adipose-derived stem cells (ADSC) were transfected using the Neon transfection system and 0.5  $\mu\text{g}$  of a plasmid encoding the EGFP. 48 hours post transfection, the cells were analyzed by (A) light and (B) fluorescence microscopy.

**Table 5. 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 the live versus dead cells.

### Ordering information

| Product   | Quantity      | Cat. No. |
|---|---------------|----------|
| Neon Transfection System Starter Pack             | 1 pack        | MPK5000S |
| Neon Transfection System                          | 1 each        | MPK5000  |
| Neon Transfection System Kit (100 $\mu\text{L}$ ) | 192 reactions | MPK10096 |
| Neon Transfection System Kit (100 $\mu\text{L}$ ) | 50 reactions  | MPK10025 |
| Neon Transfection System Kit (10 $\mu\text{L}$ )  | 192 reactions | MPK1096  |
| Neon Transfection System Kit (10 $\mu\text{L}$ )  | 50 reactions  | MPK1025  |
| Neon Transfection System Pipette                  | 1 each        | MPP100   |
| Neon Transfection System Pipette Station          | 1 each        | MPS100   |
| Neon Transfection Tubes                           | 1 pack        | MPT100   |

## Lipofectamine transfection reagents

Invitrogen™ Lipofectamine™ transfection reagents are the most trusted and cited in the scientific literature due to their superior transfection performance and broad cell spectrum. An overview of our most effective transfection products for MSCs are shown to help you choose the solution that's right for you.

**Table 6. Transfection reagent selection guide.**

| Transfection product  | DNA | mRNA | RNAi | Co-delivery | Performance | Ease of use | Price | Considerations     |
|---|-----|------|------|-------------|-------------|-------------|-------|--------------------|
| <b>Gene expression</b>  |     |      |      |             |             |             |       |                    |
| Invitrogen™ Lipofectamine™ 3000 Transfection Reagent          | ✓   |      | ✓    | ✓           | ***         | ****        | *     |                    |
| Invitrogen™ Lipofectamine™ MessengerMAX™ Transfection Reagent |     | ✓    |      |             | ****        | ***         | **    | Need to make mRNA  |
| <b>Downregulation</b>   |     |      |      |             |             |             |       |                    |
| Invitrogen™ Lipofectamine™ RNAiMAX™ Transfection Reagent      |     |      | ✓    |             | ***         | ****        | **    | Need to lift cells |

### Lipofectamine 3000 Reagent



Versatile reagent with the power to transfect your most difficult cells

- Superior efficiency—into the broadest spectrum of difficult-to-transfect cells
- Gentle with low toxicity—for improved cell viability
- Versatile—single kit for DNA, RNA, and cotransfection

### Lipofectamine MessengerMAX Reagent



mRNA transfection reagent with up to 5x the efficiency of DNA reagents

- Amazing transfection efficiency in neurons and primary cell types
- Faster protein expression with no risk of genomic integration
- Up to 10x higher cleavage efficiency using mRNA CRISPRs

### Lipofectamine RNAiMAX Transfection Reagent



Advanced, efficient solution for siRNA delivery

- Easy and efficient siRNA delivery in a wide variety of cell lines
- Unmatched performance, delivering greater knockdown with less siRNA
- Easy optimization with a simple protocol

### Ordering information

| Product                                    | Quantity | Cat. No. |
|--|----------|----------|
| Lipofectamine 3000 Transfection Reagent    | 0.75 mL  | L3000008 |
| Lipofectamine 3000 Transfection Reagent    | 1.5 mL   | L3000015 |
| Lipofectamine MessengerMAX Reagent         | 0.3 mL   | LMRNA003 |
| Lipofectamine MessengerMAX Reagent         | 0.75 mL  | LMRNA008 |
| Lipofectamine RNAiMAX Transfection Reagent | 0.75 mL  | 13778075 |
| Lipofectamine RNAiMAX Transfection Reagent | 1.5 mL   | 13778150 |

# MSC characterization

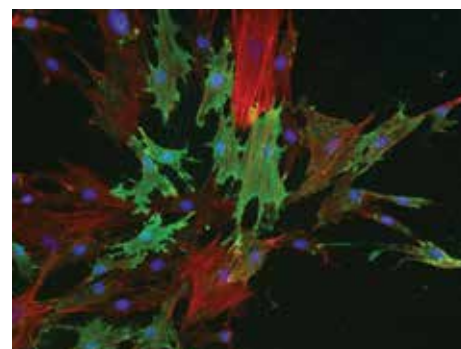
## MSC primary antibodies

We offer a comprehensive library of primary antibodies for mesenchymal stem cells (Figures 25 and 26). This library includes 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. Each antibody is of high quality and has been extensively tested and validated. We also offer custom conjugation of any antibody to the fluorophore of your choice.

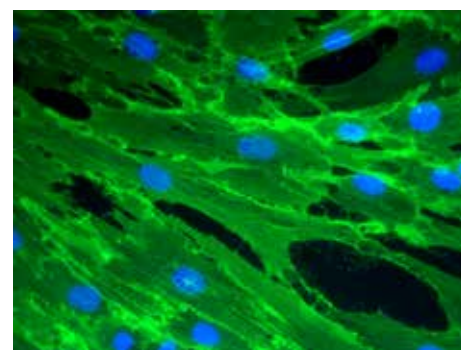
Find out more at [thermofisher.com/antibodies](https://thermofisher.com/antibodies)

### Ordering information

| Product  | Cat. No. (Antibody clone ID)   |
|--|--|
| <b>MSC primary antibodies – positive markers</b> |  |
| CD73   | 410200 (7G2), MA515537 (1D7)   |
| CD90 (Thy-1)                                     | MA516683 (FF-10), MA517747 (5a-8), MA517752 (IBL-1)  |
| CD105  | MA511854 (SN6h), MA517041 (3A9), MA119231 (MEM-226), MA119408 (MEM-229), PA516895, PA127884, MHCD10500 (SN6)                               |
| CD44   | 701406 (19H8L4), 710413 (19HCLC), MA512394 (5F12), MA513887 (156-3C11), MA513890 (156-3C11), MHCD4401 (MEM-85), MHCD4404, RM5704 (IM7.8.1) |
| CD36   | MA514112 (185-1G2), PA116813, MA119407 (TR9), MA516941 (SMØ)   |
| Nestin   | MA1110 (10C2)  |
| Stro-1   | 398401 (STRO-1)  |
| <b>MSC primary antibodies – negative markers</b> |  |
| CD11b  | CD11b00 (VIM12), RM2804 (M1/70.15)   |
| CD14   | MHCD1400 (Tük4), Q10053 (HIT2), MA511394 (7)   |
| CD19   | AHS1912 (SJ25-C1), Q10379 (6D5)  |
| CD34   | RM3604 (MEC 14.7), 180227 (BI-3C5), CD3458101 (581)  |
| CD79a  | MA511636 (JCB117), MHCD79a04 (HM47), MA513212 (HM47/A9), MA514556 (SP18), MA515234 (2F11)  |
| CD45   | 180367 (MEM28/MEM56/MEM55), CD11208 (HI30), MA512795 (Bra55), MA513197 (PD7/26/16 + 2B11), MCD4500 (30-F11) MHCD4501 (HI30), MR6918 (OX-1) |
| HLA-DR   | H11212 (Tü36)  |



**Figure 25. Positive marker for mesenchymal stem cells.** Immunofluorescence analysis of mesenchymal stem cells using the mouse anti-Stro-1 (Cat. No. 39-8401) and goat anti-mouse Alexa Fluor™ 488 (Cat. No. A21042) (green). Actin is stained with Alexa Fluor™ 594 phalloidin (red) and nuclei are stained with DAPI (blue). Sample is mounted in ProLong™ Gold antifade reagent.



**Figure 26. Immunofluorescence analysis of human mesenchymal stem cells cultured under basal conditions using anti-CD105 antibody (Cat. No. MHCD10500).** Nuclear DNA was stained with DAPI (blue).

## Qtracker cell labeling kits

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

Invitrogen™ Molecular Probes™ Qtracker™ Cell Labeling Kits deliver fluorescent Qdot™ nanocrystals (Figure 27) 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 cell generations and are not transferred to adjacent cells in a population.

Find out more at

[thermofisher.com/qdots](http://thermofisher.com/qdots)

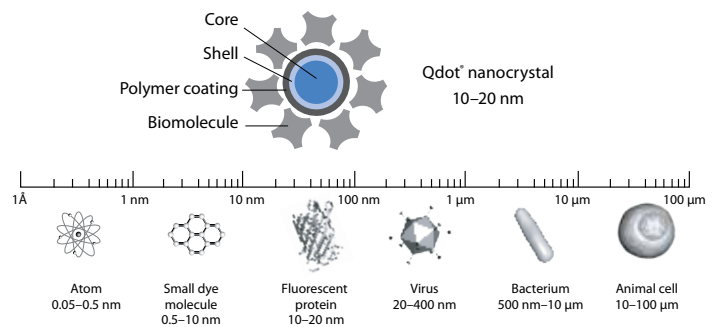
## TaqMan Gene Expression Assays

We offer more than 1.3 million Applied Biosystems™ TaqMan™ Gene Expression Assays, a comprehensive set of pre-designed real-time PCR assays (Figure 28). All TaqMan Gene Expression Assays have been designed using our validated bioinformatics pipeline, and run with the same PCR protocol, eliminating the need for primer design or PCR optimization.

Below are the matched gene expression markers of 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.

### Ordering information

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



**Figure 27. 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. |
|------------------------------------|----------|----------|
| <b>Qtracker Cell Labeling Kits</b> |          |          |
| 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 |



**Figure 28. TaqMan Gene Expression Assays.**

## TaqMan Gene Expression Arrays (plates and cards)

TaqMan Array cards and plates contain TaqMan Gene Expression Assays dried down in 384-well TaqMan Array microfluidic cards and 96-well (standard or Fast) TaqMan Array plates. They are suitable for gene expression screening and validation applications that require the analysis of tens to hundreds of targets. TaqMan Arrays are available in various formats help to meet your laboratory's needs.

- Preconfigured (fixed content)—choose premade arrays that contain the most widely used predefined gene expression panels (categorized by specific disease, pathway, or biological process). Some examples of TaqMan Arrays relevant for MSC characterization are listed below.
- Flexible content—select preconfigured pathway panels (human, mouse, and rat) and modify assay content to suit your needs. Examples include:
  - Hypoxia signaling pathway
  - Mesenchymal stem cell
  - Stem cell signaling
  - Stem cell transcription factors ChIP
  - Stem cells

Visit [thermofisher.com/flexiblepanels](https://thermofisher.com/flexiblepanels)

### Ordering information

| Product                                       | Quantity | Cat. No. (Standard 96-well) | Cat. No. (Fast 96-well) |
|---|----------|-----------------------------|-------------------------|
| TaqMan Array Human Cell Surface Markers Plate | 1 plate  | 4414109                     | 4418754                 |
| TaqMan Array Human BMP Pathway Plate          | 1 plate  | 4414110                     | 4418755                 |
| TaqMan Array Human Hypoxia Plate              | 1 plate  | 4414090                     | 4418735                 |
| TaqMan Array Human Osteogenesis Plate         | 1 plate  | 4414096                     | 4418741                 |

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# Cell Therapy Systems

As you move from basic cell therapy research to the clinic, high-quality products and proper documentation are essential to getting it right the first time. CTS products help minimize the risk of contamination and variability in your research and provide all the required documentation for regulatory review—making them the superior choice as you transition from the bench to the clinic.

The Cell Therapy Systems brand offers a broad array of high-quality products designed for use in cell therapy research applications, including media, reagents, growth factors, enzymes, selection beads, and devices, which are all manufactured in compliance with 21 CFR Part 820 Quality System Regulation and/or are certified to ISO 13485 and ISO 9001.

When you choose the CTS brand, you can expect:



## Harmonized documentation

- Traceability documentation including certificates of analysis, certificates of origin and drug master files
- Helps reduce time in preparing investigational new drug (IND) submission
- All products are ready to be used as part of your IND (CMC-ready)
- CTS product labeling and intended use statements
- cGMP—and FDA-compliant product formulations



## Seamless transition from research to clinic

- Defined formulations minimize lot-to-lot variability
- Manufactured under scalable cGMP conditions
- Complementary RUO products
- Extensive QA testing for sterility, endotoxin, adventitious agents, and mycoplasma on most products



## Expert consultation

- Regional technical support for all CTS products
- Experienced global professionals to help navigate regulatory processes from research to commercial phase
- Cell therapy expertise to help answer all of your questions
- CTS brand is backed by more than 50 years of Gibco media experience

To learn more about CTS products offered, visit [thermofisher.com/celltherapy](https://thermofisher.com/celltherapy)

# Custom culture media

## 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 29).

- Liquid in batches from 10 liters to 10,000 liters
- 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 30)

#### 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 scale (Figure 31).

#### Gibco™ MediaExpress™ and Rapid Research services

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 Gibco™ Custom Services team to minimize process development inefficiencies and help improve time and cost performance using our latest technologies.

#### cGMP manufacturing sites

We maintain two primary Gibco cell culture manufacturing locations—USA and Scotland—and three primary Gibco sera and/or protein products manufacturing locations—USA, New Zealand, and Australia. For reliable global service and contingency planning, we welcome visits and audits to 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.

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Figure 29. cGMP manufacturing sites.

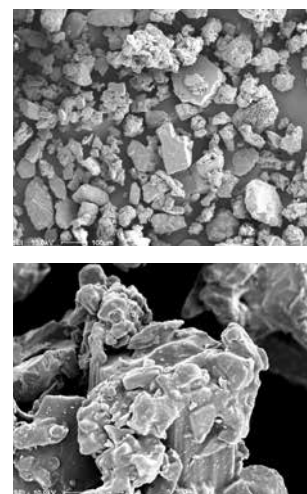


Figure 30. Advanced granulation technology (AGT) media.



Figure 31. Custom packaging options.

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## StemPro MSC SFM XenoFree, StemPro MSC SFM, MesenPRO RS and MSC-Qualified FBS

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### (adipogenesis, chondrogenesis, and osteogenesis)

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## StemPro Alk Phos Expressing Rat MSC

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## Additional resources for MSC research

31. Mesenchymal Stem Cell Assays and Applications, Series: *Methods in Molecular Biology*, Vol. 698, Vemuri MC, Chase LG, Lipnick S (Eds.) 2011.
32. Mesenchymal Stem Cell Therapy, Series: *Stem Cell Biology and Regenerative Medicine*, Chase LG, Vemuri MC (Eds.) 2013.

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