



Human Platelet Lysate (hPL) PLATINUM

Reference number: P480C

Storage temperature: $\leq -70^{\circ}\text{C}$

No heparin addition required

Xeno-free

Clinical grade

Product description

hPL is a growth factor-rich cell culture supplement derived from human blood donations. Each batch of hPL contains material from maximally 480 donors.

The product is fibrinogen depleted and does not contain heparin.

This hPL was manufactured under a quality management system based on ISO-13485. The principles, recommendations and requirements of ISO-20399 and of the European Pharmacopoeia (Chapter regarding raw materials of biological origin for the production of cell-based and gene therapy medicinal products) have been applied.

See also the enclosed Certificate of Analysis.

Instructions for use

Storage/Stability

This product should be stored at $\leq -70^{\circ}\text{C}$ upon receipt. Thaw at room temperature or in a water bath $\leq 37^{\circ}\text{C}$. After thawing, aliquot at will and store aliquots at -20°C or colder. It is recommended to store the products at -15°C to -30°C for no more than 8 weeks. For long term storage, temperatures $\leq -70^{\circ}\text{C}$ are recommended.

Multiple freeze/thaw cycles may lead to increased turbidity and lower efficiency.

The expiry date of the product is indicated on the product label.

Preparation

For use in cell culture media, we recommend an optimal dilution of 10% (vol/vol) in basal medium. In rare cases, a concentration of hPL $> 10\%$ (vol/vol) may cause salt precipitation if used in basal media containing high phosphate concentrations (e.g. RPMI) combined with certain cell types. Filtration of complete media $< 0.4\mu\text{m}$ is recommended. For assistance in selecting your basal media, contact trec@rodekruis.be.



Precautions and safety

- Not intended for direct use in animals or humans.
- Apply GLP according to biosafety regulations.

Because these products contain human derived materials, they may carry a risk of transmitting infectious agents.

The hPL is prepared from blood donations collected from consenting, non-remunerated voluntary donors of the Belgian Red Cross Flanders.

The source products for production of hPL are prepared according to Belgian legislation. With each donation, the donor is approved by a physician (based on a medical questionnaire) and tested for antibodies against the human immunodeficiency viruses (anti-HIV-1 and HIV-2), for antibodies against the hepatitis C virus (anti-HCV), for hepatitis B virus surface antigen (HBsAg) and for antibodies against *Treponema pallidum*. HIV, HBV and HCV are also detected by NAT testing.

The hPL production process includes a pathogen inactivation step (using the INTERCEPT method from Cerus) on the final product. This reduces the risk of transmission of HIV, HCV and HBV to almost zero. Due to its broad spectrum of action, pathogen inactivation also significantly protects against transmission of less or unknown pathogens ("emerging infectious diseases"). However, such transmission can never be excluded.

Prions are not eliminated by pathogen inactivation and cannot be detected in donors by routine lab tests. Protection against transmission of prions by transfusion relies on careful donor selection.

Although these donor screening, testing and pathogen inactivation procedures are in place to minimize the risk of transmitting infections, they cannot completely eliminate this risk. Therefore the products should be handled and treated as potentially infectious.

Reporting of complaints regarding the product or service

Customer satisfaction is of primary importance. We therefore consider it important that complaints or comments regarding the products or services are always reported to us. In this way, these complaints can be followed up and can lead to improvement.

Please report any complaints or comments to tinc@rodekruis.be.





Human Platelet Lysate (hPL) GOLD

Reference number: G150C

Storage temperature: $\leq -70^{\circ}\text{C}$

No heparin addition required

Xeno-free

Clinical grade

Product description

hPL is a growth factor-rich cell culture supplement derived from human platelet concentrates. Each batch of hPL contains material from maximally 150 donors.

The product is fibrinogen depleted and does not contain heparin.

This hPL was manufactured under a quality management system based on ISO-13485. The principles, recommendations and requirements of ISO-20399 and of the European Pharmacopoeia (Chapter regarding raw materials of biological origin for the production of cell-based and gene therapy medicinal products) have been applied.

See also the enclosed Certificate of Analysis.

Instructions for use

Storage/Stability

This product should be stored at $\leq -70^{\circ}\text{C}$ upon receipt. Thaw at room temperature or in a water bath $\leq 37^{\circ}\text{C}$. After thawing, aliquot at will and store aliquots at -20°C or colder. It is recommended to store the products at -15°C to -30°C for no more than 8 weeks. For long term storage, temperatures $\leq -70^{\circ}\text{C}$ are recommended.

Multiple freeze/thaw cycles may lead to increased turbidity and lower efficiency.

The expiry date of the product is indicated on the product label.

Preparation

For use in cell culture media, we recommend an optimal dilution of 10% (vol/vol) in basal medium. hPL Gold is validated for use with DMEM/F-12, RPMI, Ham's F-12 and IMDM. Combination of hPL Gold with DMEM, MEM or similar are likely to cause precipitation and issues in your cell culture. If the use of DMEM or MEM is required, hPL Platinum might be a better fit. For assistance in selecting your basal media, contact trec@rodekruis.be.

Filtration of complete media $<0.4\mu\text{m}$ is recommended.



Precautions and safety

- Not intended for direct use in animals or humans.
- Apply GLP according to biosafety regulations.

Because these products contain human derived materials, they may carry a risk of transmitting infectious agents.

The hPL is derived from human platelet concentrates prepared from donations collected from consenting, non-remunerated voluntary donors of the Belgian Red Cross Flanders. With each donation, the donor is approved by a physician (based on a medical questionnaire) and tested for antibodies against the human immunodeficiency viruses (anti-HIV-1 and HIV-2), for antibodies against the hepatitis C virus (anti-HCV), for hepatitis B virus surface antigen (HBsAg) and for antibodies against *Treponema pallidum*. HIV, HBV and HCV are also detected by NAT testing.

The platelet concentrates are prepared according to Belgian legislation, including pathogen inactivation.

Prions are not eliminated by pathogen inactivation and cannot be detected in donors by routine lab tests. Protection against transmission of prions by transfusion relies on careful donor selection.

Although these donor screening, testing and pathogen inactivation procedures are in place to minimize the risk of transmitting infections, they cannot completely eliminate this risk. Therefore the products should be handled and treated as potentially infectious.

Reporting of complaints regarding the product or service

Customer satisfaction is of primary importance. We therefore consider it important that complaints or comments regarding the products or services are always reported to us. In this way, these complaints can be followed up and can lead to improvement.

Please report any complaints or comments to tinc@rodekruis.be.





PLTGold® Gamma Irradiated (PLTGold®-GI) Product Insert

About PLTGold®-GI

PLTGold®-GI is a non-xenogeneic, animal serum-free product derived from human platelets. PLTGold®-GI is used as a manufacturing component in the generation of adult stems cells. A Master File for PLTGold®-GI is registered with the FDA and is cross-referenceable. Contact us for more information on the MF.

Product	Catalog Number	Size
PLTGold®-GI Clinical Grade (GMP)	PLTGold27GMP-GI	27mL
	PLTGold100GMP-GI	100mL
	PLTGold500GMP-GI	500mL
	PLTGold1000GMP-GI	1,000mL

Safety Information & Precautions

- Products not intended for direct use in animals or humans.
- All PLTGold®-GI donors have been tested for infectious diseases. In addition, the final product has been gamma irradiated. However, as a blood derived product, PLTGold®-GI should be handled and treated as potentially infectious.
- Universal precautions for handling and disposal of biological products should be used when working with PLTGold®-GI.

Using PLTGold®-GI

- Thaw at 37°C or 4°C.
- It is not recommended to expose PLTGold®-GI to repeated temperature changes that could affect the integrity of its components. For that reason, we recommend thawing the product and preparing aliquots as soon as it is received.
- Aliquots can be stored at -20°C or colder protected from light. Storage at 4°C is recommended for periods no longer than 2 weeks.
- Some turbidity and/or protein aggregates may appear with PLTGold®-GI. This is normal due to the nature of the product.
- Filtration of PLTGold®-GI by itself is not recommended. Filtration of complete media containing PLTGold®-GI may be performed, if necessary.

Culture Conditions Using PLTGold®-GI

- Cell seeding should be performed following the general guidelines for the specific cell type. For Mesenchymal Stem Cells (MSCs), cells are typically plated at approximately 2×10^3 – 5×10^3 cells per cm^2 .
- For MSCs, PLTGold®-GI can be used at 5% vol/vol in a typical cell culture medium such as DMEM or α -MEM. If the basic media doesn't contain Glutamine, a source of L-Glutamine will need to be added to the media at a final concentration of 2mM. For other types of cells, the concentration of PLTGold®-GI will need to be titrated according to the application (a titration from 2% vol/vol to 10% vol/vol is recommended to establish the percentage of PLTGold®-GI needed for the cell type to use).
- Do not allow primary MSC confluence to exceed 70-80%.

Origin

- PLTGold®-GI was developed to provide a gamma irradiated hPL. It is derived from our current product, PLTGold®.

References

- Crespo-Diaz R, Behfar A, Butler GW, et al. **Platelet lysate consisting of a natural repair proteome supports human mesenchymal stem cell proliferation and chromosomal stability.** *Cell Transplant.* 2011;20(6):797-811.
- Burnouf T, Strunk D, Koh MB, et al. **Human platelet lysate: Replacing fetal bovine serum as a gold standard for human cell propagation?** *Biomaterials.* 2016 Jan;76:371-87.
- Alonso-Camino V, Clarke B, Nielsen J, et al. **In vitro expansion of mesenchymal stem cells using media supplemented with unfractionated heparin-free platelet lysate.** Poster presented at: ISCT Annual Meeting. London, UK. 2017 May.
- Bulur P, Wiltshire T, Dudakovic A, et al. **Impact of media supplementation on the secretion of IFN- γ induced indoleamine 2-3 deoxygenase and resultant immune suppression by mesenchymal stromal cells.** Poster presented at: ISCT Annual Meeting. Montreal, Canada. 2018 May.
- Alonso-Camino V, Mirsch W. **In vitro expansion of human primary endothelial cells for clinical use using EndoGo™ XF Medium supplemented with PLTGold® human platelet lysate.** Poster presented at: ISCT Annual Meeting. Montreal, Canada. 2018 May.

Manufacturer:

Mill Creek Life Sciences, LLC, 221 1st Avenue SW, Ste 209, Rochester, MN 55902 USA

For Technical and Ordering Information, Please Contact Us At:

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nLiven PR™

As global regulatory expectations evolve, Sexton Biotechnologies is there to meet the challenge. With a validated pathogen reduction technology, nLiven PR established the gold standard for performance and quality of irradiated pooled human platelet lysate.

▲ Specification

- Validated pathogen reduction technology using E-Beam irradiation
- Available in 100, 500 ml
- Large donor pool lot sizes
- Manufactured from outdated FDA/AABB approved apheresis platelet units
- FDA master file, PMDA Certificate of Suitability
- Used for manufacturing in US, EU, and other clinical trials globally
- Manufactured under a certified ISO 9001:2015 Quality Management System
- Example CoA and IFU

▲ Details

- Culture supplement to replace FBS or AB Serum
- BioDefined composition reduces lot-to-lot variability
- Supports a wide range of cell types including MSCs, T-Cells, NK Cells, Endothelial cells, and more.
- Promotes improved in vitro and In vivo cell quality in CAR-T applications
- Typically used at 2%-10% concentrations.
- Used in 2D and 3D expansion systems
- Additional pathogen reduction applied per EU pharmacopeia

Please download **nLiven PR™** brochure here:

- **nLiven PR™** (https://www.biolifesolutions.com/wp-content/uploads/2023/03/NLiven_v3-copy.pdf)

Frequently

Human Platelet Lysate (Stemulate®) Pathogen-Reduced Human Platelet Lysate (nLiven PR™ and T-Liven PR™) Product Instructions

Product Description:

Stemulate®, nLiven PR™, T-Liven PR™ are cell culture supplements derived from human platelets.

- Product Codes: PL-SP-100, PL-SP-500, PL-NH-100, PL-NH-500, PL-PR-100, PL-PR-500, TL-PR-50C, TL-PR-100C, TL-PR-150C, TL-PR-250C, TL-PR-500C
- Heparin was not added at any point during manufacture and is not required for use of this product.

Precautions:

Universal precautions should be used in the handling and disposal of Stemulate, nLiven PR, and T-Liven PR.

Instructions for Use

Receipt and Storage:

This product is supplied sterile filtered and aseptically filled. Sterility is maintained if container is not opened and not damaged. Recommended storage of this product is -20°C.

Thawing Instructions:

For bottled product, place the container in a 37°C water bath until the product has no ice remaining. It is not recommended to thaw the product on a bench top or in a refrigerator. Once thawed, the product may be used immediately.

For bagged product, first ensure that the overpack bag is completely sealed. If possible, submerge only the portion of the bag containing the product below the water surface; keep the opening of the overpack bag/tubing above the water surface. Thaw the product until no ice remains. It is not recommended to thaw the product on a bench top or in a refrigerator. Once thawed, the product may be used immediately.

Appearance:

Turbidity or precipitate may appear upon thawing. This precipitate may also appear during short term storage or prolonged storage within a buffered system at a refrigerated temperature. This is normal and will not affect performance of the product. Filtration is not recommended.

Stability:

Thaw and aliquot the product upon receipt to minimize freeze/thaw cycles. For short term storage up to 24 hours, the product must be stored at a temperature between 2-8°C.

Preparation and Use:

Optimal concentration of this product must be determined for each cell line and/or application.



Advanced Cell-Based Solutions & Services



Human Platelet Lysate



Human Platelet Lysate

Human Platelet Lysate (HPL) is a non-animal derived cell culture growth supplement obtained from blood platelets after freezing and thawing. HPL contains abundant growth factors and cytokines necessary for cell growth and proliferation. Platelet lysate can be used as a source of growth factors to replace bovine serum in cell culture medium. For example, growth kinetics for primary kidney cells maintained in 5% HPL-containing medium were found to be comparable to that of cultures grown in medium supplemented with 10% FBS. Replacing bovine serum with HPL allows for expansion and clinical grade production of functional mesenchymal stromal cells by removing the risk of xenogeneic immune reactions and transmission of bovine prion and viral pathogens.

The US FDA is moving towards adopting European guidelines requiring that bovine materials be removed from culturing media for any therapeutic product containing cultured cells, thus making HPL an attractive alternative to the production and manufacturing processes. **All lots are fully tested for pathogens according to FDA guidelines. ZenBio HPL is for research purposes only.**

See performance data on the back—>

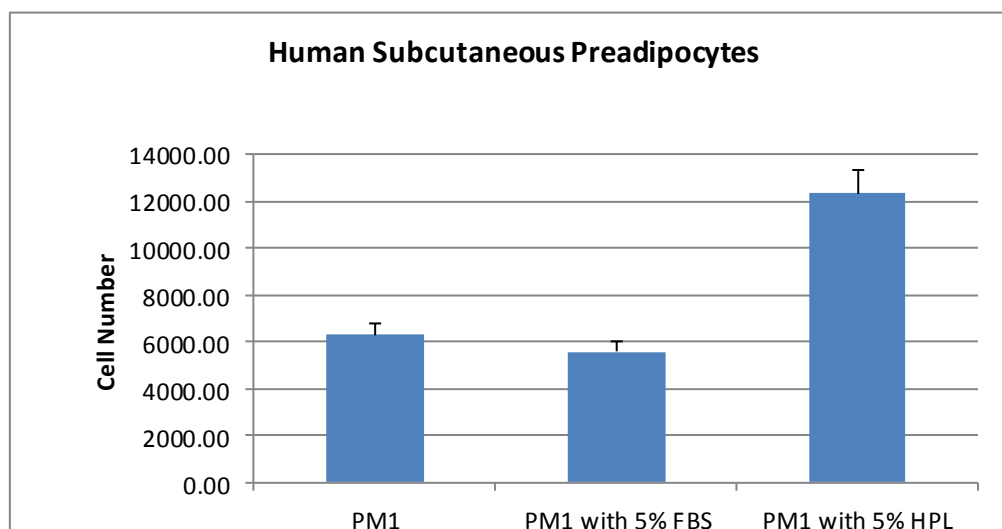
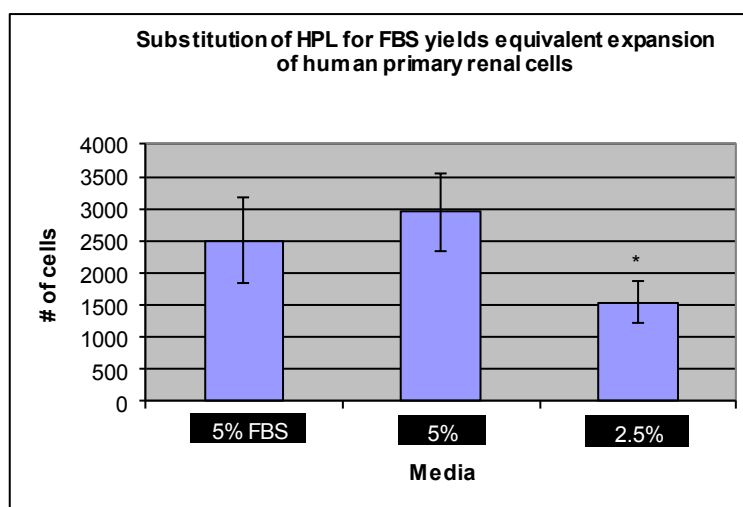
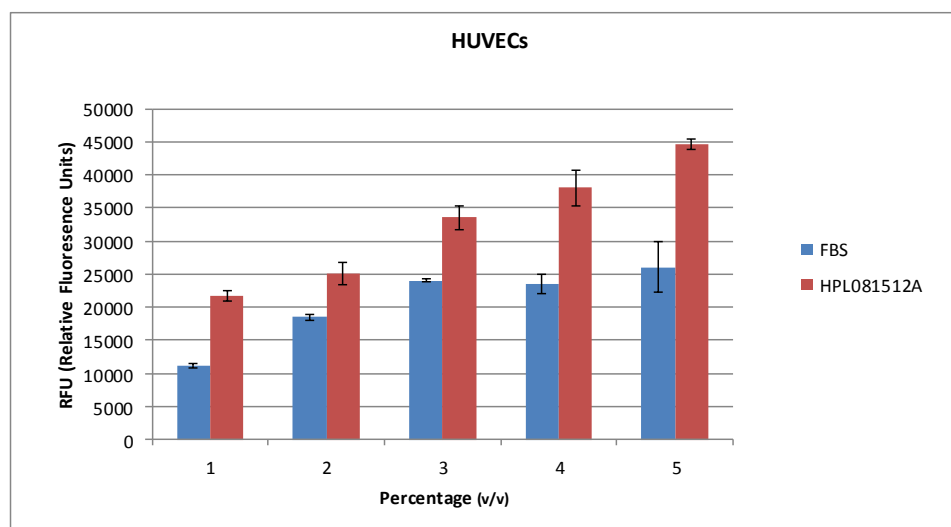
Product Description	Size/Qty	Cat. #	Price
Human Platelet Lysate, Frozen, Pooled	100ml	SER-HPL	\$325.00



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Characterized Fetal Bovine Serum, US Origin

HYCLONE SERA

HyClone™ Characterized Fetal Bovine Serum (FBS) provides important proteins, hormones, growth factors, metabolites, and nutrients for your cell culture process (Fig 1). Characterized FBS is produced using true pool technology, which minimizes bottle-to-bottle variability.

Key features of Characterized FBS include

- Sourced in the USA
- Complete traceability back to original source
- Low in antibodies and high in growth factors
- Produced using true pool technology for uniformity and consistency from bottle to bottle

Specifications

Characterized FBS is filtered through three sequential 100 nm (0.1 µm) pore size-rated filters. Before dispensing, each lot of serum is pooled to ensure uniformity and consistency between bottles (true pool technology).

Characterized FBS is assayed for gamma globulin, alkaline phosphatase, lactate dehydrogenase, glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, pH, total protein, albumin, blood urea nitrogen, creatinine, total bilirubin, sodium, potassium, calcium, chloride, inorganic phosphorus, osmolality, iron, total iron binding capacity (TIBC), percent iron saturation, glucose, and IgG. Included assays are subject to change without notice.

Product handling

Storage and handling

Sera should be stored at -10°C or lower. Once thawed, sera should be stored at 2°C to 8°C. To maintain quality, a conservative storage recommendation for sera at this temperature is no longer than six weeks. If the serum needs to be stored longer than six weeks after opening, it is recommended to aliquot the serum into convenient volumes and refreeze.



Fig 1. Characterized Fetal Bovine Serum provides important proteins and nutrients to your cell culture.

Thawing

Remove serum from -10°C (or lower) storage and place in a refrigerator overnight at 2°C to 8°C. Transfer the serum to a 37°C water bath, agitate periodically to mix the solutes concentrated at the bottom of the container. Do not hold the serum at 37°C any longer than necessary after thawing. Thawing serum in a bath above 40°C without mixing can denature the concentrated proteins in the bottom of the container and precipitates might form in the bottle. Thawing serum at higher temperatures is not recommended.

Alternatively, bottles can be placed directly from -10°C (or lower) storage into a 37°C water bath. Bottles should be agitated to enhance mixing and thawing. Turbidity and flocculent material might be present after thawing or after prolonged storage.

Experience indicates that regardless of the method used to thaw serum, it is critical that it is mixed during the thawing process to prevent the formation of gradients and subsequent precipitation. Because of differences in thawing rates of different components, serum will form a gradient if it is not mixed as it thaws. If serum is allowed to remain in such a gradient state, precipitation is likely to occur. Handle bottles that have been stored in freezer carefully. Avoid large temperature shifts and protect the serum from exposure to light. Wear protective clothes and equipment. Storage requirements are listed on the product label.

General culture recommendations

We recommend supplementation of classical media such as Dulbecco's Modified Eagle's Medium (DMEM) at a range between 5% and 10% FBS to support culture of a wide variety of cell lines and applications.

Quality control testing

Quality control test specifications are listed in Table 1.

Table 1. Test specifications

Endotoxin (Limulus amebocyte lysate gel clot assay)	≤ 25 EU/mL
Hemoglobin (spectrophotometric)	< 25 mg/dL
Sterility testing (current USP)	
Bacteria and fungi	No growth
Virus Testing (9 CFR 113.53)	
Fluorescent Antibody	
Bluetongue	Not detected
Bovine adenovirus	Not detected
Bovine parvovirus	Not detected
Bovine respiratory syncytial virus	Not detected
Bovine viral diarrhea virus	Not detected
Rabies	Not detected
Reovirus	Not detected
Cytopathogenic agents (e.g., IBR)	Not detected
Hemadsorbing agents (e.g., PI3)	Not detected
Mycoplasma	
Large volume, direct culture	Not detected
Hoechst DNA stain	Not detected
Certificate of suitability	Included

Related products

HyClone classical media

Our classical media are manufactured using ISO 9001:2008- and ISO 13485:2003-certified processes. All raw material components have passed strict quality control testing to ensure the appropriate level of quality. The classical media are hydrated using water for injection grade water and have undergone 0.1 µm sterile filtration.

Classical media that have been system tested with Characterized FBS to confirm product efficacy and homogeneity are

- Dulbecco's Modified Eagle's Medium (DMEM)
- Minimal essential medium (MEM)
- Eagle's minimal essential medium (EMEM)
- RPMI 1640 medium

HyClone phosphate buffered saline (PBS)

Our PBS products are manufactured according to cGMP guidelines using ISO 9001:2000-certified processes. The products have full traceability and documented origin of all formula ingredients.

HyClone trypsin protease

Our trypsin protease is derived from porcine pancreas and is gamma irradiated prior to hydration and filling. The product is formulated without calcium and magnesium.

Ordering information

Product	Size	Product code
HyClone Characterized	50 mL	SH30071.01
Fetal Bovine Serum, US Origin	100 mL	SH30071.02
	500 mL	SH30071.03

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CY12686-18Jul20-DF



MesenCult™-ACF Plus Medium

Animal component-free medium for human mesenchymal stem cells

Catalog #05445 1 Kit
#05448 1 Kit



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Product Description

MesenCult™-ACF Plus Medium is a standardized, animal component-free (ACF) and serum-free medium for the isolation and culture of human mesenchymal stromal cells, also known as mesenchymal stem cells (MSCs) from bone marrow (BM-MSCs) and adipose tissue (ADSCs). MesenCult™-ACF Plus Medium is optimized for the expansion of MSCs in vitro as well as their enumeration using the colony-forming unit-fibroblast (CFU-F) assay. MesenCult™-ACF Plus Medium supports the isolation and long-term growth of human bone marrow- and adipose tissue-derived MSCs, and cells maintain robust multi-lineage differentiation potential in vitro.

MesenCult™-ACF Plus Medium also supports the expansion of human ES- or iPS-derived mesenchymal progenitor cells (MPCs), and is included in STEMdiff™ Mesenchymal Progenitor Kit (Catalog #05240).

MesenCult™-ACF Plus Medium must be used in conjunction with Animal Component-Free Cell Attachment Substrate (Component #07130) and Animal Component-Free Cell Dissociation Kit (Catalog #05426), providing a complete, defined ACF culture system. Components of Animal Component-Free Cell Attachment Substrate and Animal Component-Free Cell Dissociation Kit are pre-screened and tested for optimal cell adherence when cells are cultured with MesenCult™-ACF Plus Medium.

For animal component-free and optimized cryopreservation, MesenCult™-ACF Freezing Medium (Catalog #05490) is recommended for human MSCs previously cultured in MesenCult™ media, including MesenCult™-ACF Plus. For a complete list of related products, including differentiation media available, visit www.stemcell.com, or contact us at techsupport@stemcell.com.

NOTE: Complete MesenCult™-ACF Plus Medium must be supplemented with L-Glutamine (Catalog #07100); see Preparation of Reagents and Materials.

Ordering Information

PRODUCT NAME	CATALOG #	SIZE	COMPONENTS
MesenCult™-ACF Plus Medium Kit	05445	1 Kit	- MesenCult™-ACF Plus Medium - MesenCult™-ACF Plus 500X Supplement
MesenCult™-ACF Plus Culture Kit	05448	1 Kit	- MesenCult™-ACF Plus Medium - MesenCult™-ACF Plus 500X Supplement - Animal Component-Free Cell Attachment Substrate

Components

The following components are available as part of a kit (Catalog #05445 or #05448) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
MesenCult™-ACF Plus Medium	05446	500 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.
MesenCult™-ACF Plus 500X Supplement	05447	1 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
Animal Component-Free Cell Attachment Substrate	07130	1 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.

None of the above components contain antibiotics.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
0.5 mL screw cap polypropylene tubes	e.g. Sarstedt 72.785.005
L-Glutamine	07100
D-PBS (Without Ca++ and Mg++)	37350
Lymphoprep™	07801
3% Acetic Acid with Methylene Blue	07060
Polypropylene conical tubes, 15 mL and 50 mL	e.g. 38009 and 38010
Non-treated culture dish, 100 mm	e.g. 38045
Collagenase Type I (0.25%) OR Collagenase A, ACF	07902 OR 07434
Tissue culture-treated 6-well plates	e.g. 38015
Animal Component-Free Cell Dissociation Kit • ACF Enzymatic Dissociation Solution • ACF Enzyme Inhibition Solution	05426
Trypan Blue	07050

Preparation of Reagents and Materials

Complete MesenCult™-ACF Plus Medium

Use sterile technique to prepare complete MesenCult™-ACF Plus Medium (MesenCult™-ACF Plus Medium + MesenCult™-ACF Plus 500X Supplement + L-Glutamine). The following example is for preparing 500 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw MesenCult™-ACF Plus 500X Supplement on ice for 1 - 2 hours or overnight at 2 - 8°C. Mix thoroughly.
NOTE: Once thawed, use immediately or aliquot and store at -20°C. For aliquoting, use 0.5 mL screw cap polypropylene tubes. Do not exceed the shelf life of the supplement. After thawing the aliquots, use immediately. **Do not re-freeze.**
2. Add 1 mL of MesenCult™-ACF Plus 500X Supplement to 500 mL of MesenCult™-ACF Plus Medium. Mix thoroughly.
3. Add L-Glutamine to a final concentration of 2 mM. Mix thoroughly.

NOTE: If not used immediately, store complete MesenCult™-ACF Plus Medium at 2 - 8°C for up to 2 weeks. Do not exceed the shelf life of the individual components.

Coating Cultureware with Animal Component-Free Cell Attachment Substrate

Use sterile technique when coating cultureware with Animal Component-Free Cell Attachment Substrate.

NOTE: Use only tissue culture-treated cultureware.

1. Dilute Animal Component-Free Cell Attachment Substrate in D-PBS (Without Ca++ and Mg++) as follows:
 - For MSCs after isolation, dilute Attachment Substrate 1 in 300 in D-PBS.
For example, add 20 µL of Substrate to 5.98 mL of D-PBS.
 - For isolation of MSCs from bone marrow, dilute Attachment Substrate 1 in 150 in D-PBS.
For example, add 40 µL of Substrate to 5.96 mL of D-PBS.
 - For isolation of MSCs from adipose tissue, dilute Attachment Substrate 1 in 300 in D-PBS.
For example, add 20 µL of Substrate to 5.98 mL of D-PBS.
2. Gently mix the diluted substrate solution. Do not vortex.
3. Immediately use the diluted substrate solution to coat cultureware. Refer to Table 1 for recommended coating volumes.

Table 1. Recommended Volumes for Coating Cultureware with Diluted Animal Component-Free Cell Attachment Substrate

CULTUREWARE	VOLUME OF DILUTED ANIMAL COMPONENT-FREE CELL ATTACHMENT SUBSTRATE SOLUTION
6-well plate	1 mL/well
T-25 cm ² flask	2.5 mL/flask
T-75 cm ² flask	6 mL/flask

- Gently tilt the cultureware to spread the substrate solution evenly across the surface.
- Incubate at room temperature (15 - 25°C) for at least 2 hours before use. Do not let the substrate solution evaporate.
NOTE: If not used immediately, the cultureware must be sealed to prevent evaporation of substrate solution (e.g. with Parafilm®). Sealed cultureware can be stored at 2 - 8°C for up to 3 days after coating. Allow stored coated cultureware to come to room temperature for 30 minutes before proceeding to step 6.
- Gently tilt the cultureware onto one side and allow excess substrate solution to collect at the edge. Remove the excess solution using a serological pipette or by aspiration. Ensure that the coated surface is not scratched.
- Wash cultureware once, using D-PBS (e.g. use 2 mL/well if using a 6-well plate).
- Aspirate wash solution when MSCs are ready to be plated.

Directions for Use

Please read the entire protocol before proceeding. Use sterile technique when performing the following protocols:

- Isolation of Human MSCs from Bone Marrow
- Isolation of Human MSCs from Adipose Tissue
- CFU-F Assay
- Expansion of Human MSCs

A) Isolation of Human MSCs from Bone Marrow (BM)

The following protocol is for isolating MSCs from 25 mL of freshly isolated human BM using density gradient medium (Lymphoprep™) separation. If using other volumes, adjust accordingly.

- Count nucleated cells in the BM sample using 3% Acetic Acid with Methylene Blue.
- Split the BM sample into 2 x 50 mL conical tubes (i.e. 12.5 mL of BM sample per tube).
- Add 22.5 mL of room temperature (15 - 25°C) D-PBS containing 2 mM EDTA per tube.
- To each of 3 x 50 mL new conical tubes, add 17 mL of Lymphoprep™.
- Layer 23 mL of the BM suspension (from step 3) on top of the Lymphoprep™ in each tube.
- Centrifuge tubes at 300 x g for 30 minutes with the **brake off**.
- Collect the mononuclear cell (MNC) layer at the plasma:Lymphoprep™ interface and place in a single new 50 mL conical tube.
NOTE: Sometimes it is difficult to see the cells at the interface. In this case, remove some of the Lymphoprep™ along with the enriched cells in order to maximize cell recovery.
- Wash cells with cold (2 - 8°C) D-PBS containing 2 mM EDTA.
- Centrifuge the tube at 300 x g for 10 minutes with the **brake on**.
- Discard supernatant and resuspend the cell pellet in complete MesenCult™-ACF Plus Medium (e.g. 2 - 4 mL of complete medium).
- Count nucleated cells using 3% Acetic Acid with Methylene Blue.

B) Isolation of Human MSCs from Adipose Tissue

- Add 2 - 4 mL of Collagenase Type I (0.25%) to the adipose tissue in a 100 mm dish.
NOTE: To maintain an ACF workflow, use Collagenase A, ACF, prepared at 0.2% in D-PBS containing 0.2% recombinant albumin.
- Finely mince tissue with a scalpel. Transfer minced tissue to a 50 mL conical tube.
- Add 5 mL of collagenase per cm³ of tissue. Incubate in a shaking water bath or shaking incubator at 37°C for 1 hour.
For example, use 15 mL of collagenase for 3 cm³ of tissue.
- Remove tube from the water bath or incubator. Place upright for 5 minutes to allow separation of the lipid layer from the aqueous layer.
- Using a pipettor or aspirator, remove and discard the top lipid layer.
- Add D-PBS containing 1 mM EDTA and 0.2% recombinant albumin (or 2% fetal bovine serum) to reach a final volume of 50 mL.
- Centrifuge cells at 300 x g for 10 minutes with the **brake on**.

8. Discard supernatant and resuspend the cell pellet in complete MesenCult™-ACF Plus Medium.
9. Count nucleated cells using 3% Acetic Acid with Methylene Blue.

C) CFU-F Assay

The following protocol is for setting up a CFU-F assay in a 6-well tissue culture-treated plate. If using other cultureware, adjust cell numbers and volumes accordingly.

NOTE: Only use tissue culture-treated cultureware.

1. Coat wells with Animal Component-Free Cell Attachment Substrate (see Preparation of Reagents and Materials).
2. Plate cells in 2 mL of complete MesenCult™-ACF Plus Medium per coated well. Plate cells at 3 - 4 different densities for each cell type used. Refer to Table 2 for recommended cell plating densities.

Table 2: Recommended Cell Plating Densities for Setting up the CFU-F Assay

CELL TYPE	PLATING DENSITY (cells/cm ²)	EXAMPLE OF NUMBER OF CELLS PER WELL OF A 6-WELL PLATE
BM-derived MSCs	1 - 4 x 10 ⁴	1 x 10 ⁵ 2 x 10 ⁵ 3 x 10 ⁵ 4 x 10 ⁵
Adipose-derived MSCs	0.5 - 4 x 10 ³	0.5 x 10 ⁴ 1 x 10 ⁴ 2 x 10 ⁴ 4 x 10 ⁴

3. Incubate at 37°C for 10 - 15 days until colonies (> 40 cells/colony) appear in the well.
4. Perform a half-medium change on day 7 (i.e. aspirate 1 mL of medium and add 1 mL of complete MesenCult™-ACF Plus Medium per well).
5. Fix, stain, and count the CFU-F colonies.

D) Expansion of Human MSCs

The following protocol is for culturing cells in a single T-25 cm² flask. If using other cultureware, adjust cell numbers and volumes accordingly.

NOTE: Only use tissue culture-treated cultureware.

1. Coat a T-25 cm² flask with Animal Component-Free Cell Attachment Substrate (see Preparation of Reagents and Materials).
2. Seed freshly isolated cells (prepared in section A or B) in 6 mL of complete MesenCult™-ACF Plus Medium per coated flask. Recommended densities are as follows:
 - For BM-derived cells: 4 - 10 x 10⁴ freshly isolated cells/cm² (i.e. 1 - 2.5 x 10⁶ cells per T-25 cm² flask)
 - For adipose-derived cells: 6 - 10 x 10³ freshly isolated cells/cm² (i.e. 1.5 - 2.5 x 10⁵ cells per T-25 cm² flask)
3. Incubate at 37°C until cells are approximately 80% confluent. This takes approximately 9 - 14 days.

NOTE: If needed, perform a half-medium change on day 7 (i.e. aspirate 3 mL of medium and add 3 mL of complete MesenCult™-ACF Plus Medium per flask).
4. Passage cells using Animal Component-Free Cell Dissociation Kit, as follows:
 - i. Coat a T-25 cm² flask with Animal Component-Free Cell Attachment Substrate (see Preparation of Reagents and Materials).
 - ii. Warm ACF Enzymatic Dissociation Solution and ACF Enzyme Inhibition Solution to room temperature (15 - 25°C). Do not incubate at 37°C.
 - iii. Wash cells once with 2.5 mL of D-PBS.
 - iv. Add 2.5 mL of ACF Enzymatic Dissociation Solution and incubate at 37°C for 3 - 6 minutes. Tap the flask to detach cells. If less than 90% of cells have detached, incubate at 37°C for an additional 1 - 2 minutes and tap the flask again.
 - v. Add 2.5 mL of ACF Enzyme Inhibition Solution and collect cells in a 15 mL conical polypropylene tube.
 - vi. Wash the flask with 5 mL of complete MesenCult™-ACF Plus Medium and place into the tube from step v.
 - vii. Centrifuge the tube at 300 x g for 8 minutes with the **brake on**.
 - viii. Discard the supernatant and resuspend the cell pellet in complete MesenCult™-ACF Plus Medium (see Notes and Tips).
 - ix. Count viable cells using Trypan Blue and a hemocytometer. Seed a coated T-25 cm² flask (prepared in step i) at a density of 1.5 - 4 x 10³ cells/cm² (i.e. 4 - 10 x 10⁴ cells/flask).

- x. Incubate at 37°C until the cells are approximately 80% confluent. This takes approximately 3 - 6 days.
- 5. Repeat step 4 as needed.

Notes and Tips

- The use of polypropylene tubes (e.g. Catalog #38009 and #38010) during subculture will help to prevent the MSCs from sticking to the tubes.
- To assess trilineage potential, MesenCult™ media are available for adipogenic differentiation (Catalog #05412), osteogenic differentiation (Catalog #05465), and chondrogenic differentiation (Catalog #05455).
- To break apart cell aggregates, use a 1 mL pipettor to gently pipette the cell pellet up and down a few times [section D, step 4 (viii)].

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Miltenyi Biotec

MSC-Brew GMP Medium

2000 mL
500 mL

Order no. 170-076-325
Order no. 170-076-326

Contents

1. Description
2. Applications
3. Warnings and precautions
4. Instructions for use
5. Glossary of symbols

1. Description

Components	MSC-Brew GMP Basal Medium, 2000 mL bag (#170-076-322) 4× 5.0 mL MSC-Brew GMP Supplement I 100× (vial) (#170-076-323) 4× 5.0 mL MSC-Brew GMP Supplement II 100× (vial) (#170-076-320) MSC-Brew GMP Basal Medium, 500 mL bag (170-076-315) 1× 5.0 mL MSC-Brew GMP Supplement I 100× (vial) (170-076-323) 1× 5.0 mL MSC-Brew GMP Supplement II 100× (vial) (170-076-320)
Main components	Salts, amino acids, vitamins, buffers, proteins
Human Proteins	Contains human albumin (pharmaceutical grade).
Specifications	For detailed specifications of the MSC-Brew GMP Basal Medium and the MSC-Brew GMP Supplements I and II please refer to the respective Product Quality Certificate (PQC). Functionality assay: Isolation and cultivation of mesenchymal stromal or stem cells (MSCs) from human bone marrow by plastic adherence (CFU-F assay) and subsequent expansion of MSCs.
Total viable aerobic count	No microbial growth detectable.
Transport	MSC-Brew Basal Medium at +2 °C to +8 °C (+36 °F to +46 °F), MSC-Brew Supplement I and MSC-Brew Supplement II at -20 °C (-4 °F).

Storage

Store MSC-Brew GMP Basal Medium protected from light at +2 °C to +8 °C (+36 °F to +46 °F). Store MSC-Brew GMP Supplement I and MSC-Brew Supplement II at -20 °C (-4 °F).

Shelf life

The use-by date is indicated on the bag and vial labels.

Disclaimer

MACS GMP Products are for *ex vivo* cell processing only, and are not intended for human *in vivo* applications. For regulatory status in the USA, please contact your local representative.

This product contains human source material and must be treated as potentially infectious. One confidential component is derived from human blood and is tested at the donor level and found nonreactive for HIV-I/II, HIV-NAT, HTLV I/II, HB Ag, HBc, HBV-NAT, HCV, HCV-NAT, RPR or FTA-ABS (Syphilis), WNV-NAT, T.cruzi.

Quality statement

MACS GMP Products are manufactured and tested under a quality management system (ISO 13485) and are in compliance with relevant GMP guidelines. They are designed following the recommendations of USP <1043> on ancillary materials.

The declaration of animal- or human-derived materials is given on the Certificate of Origin.

2. Applications

MSC-Brew GMP Medium has been developed for

- the isolation of MSCs without addition of AB-serum
- the efficient expansion of MSC
- MSCs from human cell sources, such as bone marrow, umbilical cord or adipose tissue

3. Warnings and precautions

- ▲ The instructions for use must be followed.
- ▲ Do not inject or infuse the product directly into humans. Not for human application.
- ▲ When using this product, the national legislation and regulations must be followed. Any application of *ex vivo* processed target cells is exclusively within the responsibility of the user.
- ▲ For single use only. Do not reuse.

- ▲ Use only if bags are undamaged.
- ▲ Use only if vials are undamaged and sealed.
- ▲ Do not use after the use-by date indicated on the bag and vial labels.

4. Instructions for use

Substrate pre-coating of the culture vessels is not required.

1. For the preparation of the MSC-Brew GMP Medium thaw the MSC-Brew GMP Supplements I and II at +2 °C to +8 °C (+36 °F to +46 °F) over night.
2. Add the complete vial (500 mL bag) / 4 vials (2000 mL bag) of thawed MSC-Brew GMP Supplements I and II to the MSC-Brew GMP Basal Medium.
3. Mix well. The medium is ready-to-use now. Store complete MSC-Brew GMP Medium protected from light at +2 °C to +8 °C (+36 °F to +46 °F).

Complete medium should be used up to 2 weeks when stored at +2 °C to +8 °C (+36 °F to +46 °F).

5. Glossary of symbols

	Manufacturer		Use-by date
	Order number		Protect from sunlight
	Part number		Phone
	Batch code		Fax
	Consult instruction for use		E-mail
	Do not use if package is damaged.		Website

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec B.V. & Co. KG makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec B.V. & Co. KG's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec B.V. & Co. KG is not liable for any property damage, personal injury or economic loss caused by the product.

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This data sheet and corresponding information as well as special protocols can be found under www.miltenyibiotec.com/msc-brew-gmp-medium.

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Products
Cell manufacturing platform / (BE-en)/products/cell-manufacturing-platform.html
MACS GMP Portfolio / (BE-en)/products/cell-manufacturing-platform/mac-gmp-portfolio.html
MACS GMP Cell Culture Media / (BE-en)/products/cell-manufacturing-platform/mac-gmp-portfolio/mac-gmp-cell-culture-media.html
MSC-Brew GMP Medium
expansion of MSCs from human bone marrow and other human tissues, such as adipose tissue and umbilical cord.

Choose your product

MSC-Brew GMP Medium, 2000 mL	MSC-Brew GMP Medium, 2000 mL (bag)	MSC-Brew GMP Medium, 500 mL (bag)	MSC-Brew
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**170-076-332**
For ex vivo cell processing only

MSC-Brew GMP Medium

[View components](#)
[Data sheet](#)

Size

500 mL (bag)

Quantity

1

<

>

800.00 EUR



Specifications

Data and Images
Resources

Specifications for MSC-Brew GMP Medium

Overview

MSC-Brew GMP Medium is an optimized and standardized medium for the generation and expansion of mesenchymal stem cells (MSCs) from bone marrow (BM) or other tissue sources. The formulation of MSC-Brew GMP Medium is designed to efficiently support the ex vivo expansion of MSCs while maintaining their differentiation potential. The medium is xeno- and serum-free and is manufactured under strictly controlled conditions using ingredients of the highest quality. MSC-Brew GMP Medium offers consistent lot-to-lot performance and optimal conditions for the expansion of MSCs.

Detailed product information

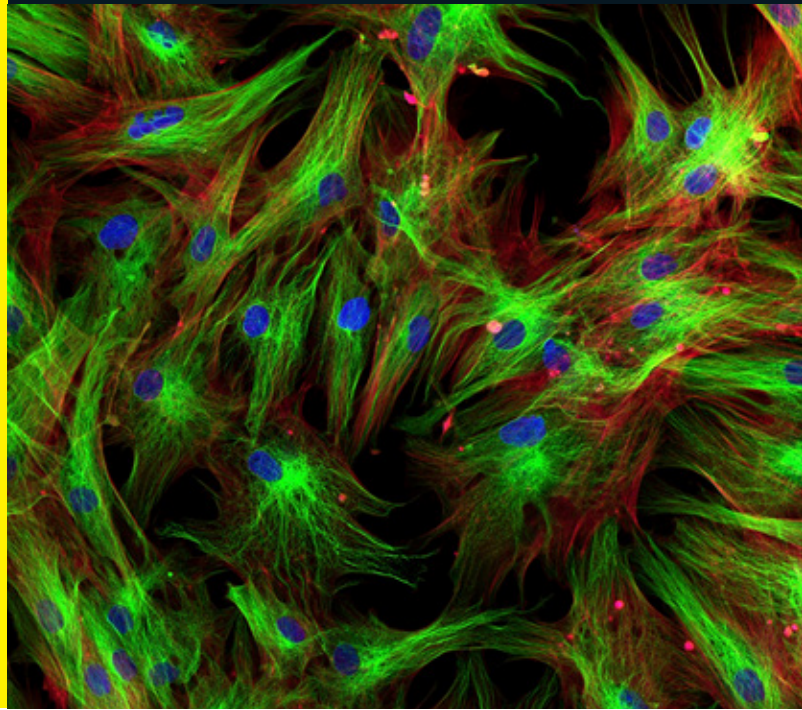
Background information

MSC-Brew GMP Medium is based on the formulation of StemMACS MSC Expansion Media Kit XF (#130-104-182) thus enabling its use in research to clinical applications.

- Serum-free and xeno-free formulation

NutriStem[®] MSC Culture System

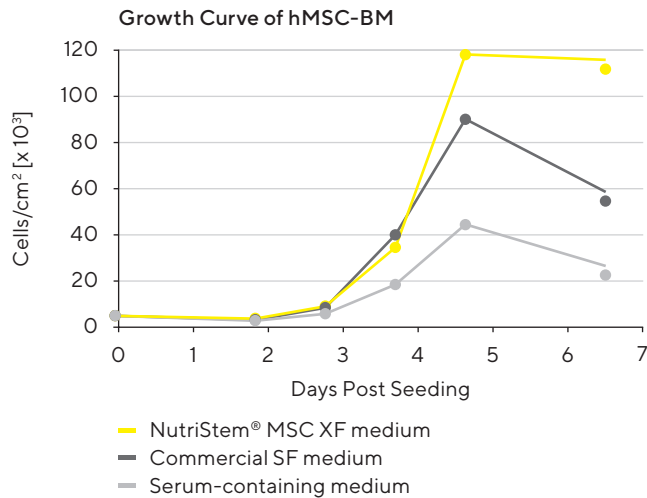
A Complete Xeno-Free,
Serum-Free System for the
Growth and Expansion of
hMSCs



Redefining Stem Cell Excellence and Advancing Clinical Applications

Defined, serum-free, xeno-free reagents are essential tools for all human mesenchymal stem cell (hMSC) research having potential clinical applications. The NutriStem[®] MSC Culture System includes defined reagents ideal for translational research use. hMSCs cultured in serum-free, xeno-free **NutriStem[®] MSC XF Medium** show superior proliferation and self-renewal potential in comparison to serum-containing media and other serum-free media.

In addition, hMSCs maintain their proper fibroblast-like cell morphology, tri-lineage differentiation potential, and demonstrate normal hMSC marker profiles and karyotypic stability over long-term culture. NutriStem[®] MSC XF Medium is designed for optimal growth and expansion of hMSCs derived from a variety of sources, including bone marrow (BM-hMSC), adipose tissue (AT-hMSC), Wharton's jelly (WJ-hMSC), placental tissue (PT-MSC), and umbilical cord matrix (UC-hMSC).



- Defined, xeno-free, serum-free medium
- Superior proliferation of hMSCs
- Supports long-term growth and differentiation potential
- FDA Drug Master File

MSC Attachment Solutions

- Xeno-free, purified human fibronectin | human fibrinogen
- Optimized for serum-free cultures
- For hMSC proliferation and differentiation

MSC Dissociation Solutions

- Ready-to-use, defined
- Recombinant trypsin solutions

NutriFreez® D10 Cryopreservation Solution

- Chemically defined, animal component-free, protein-free
- Excellent cell attachment and viability

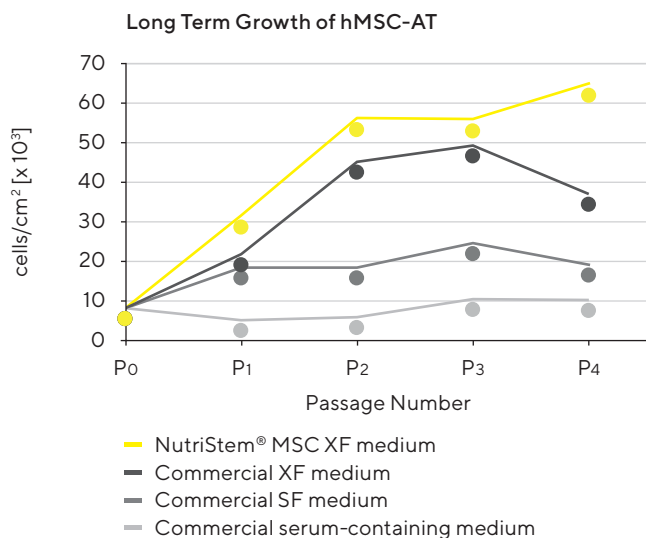
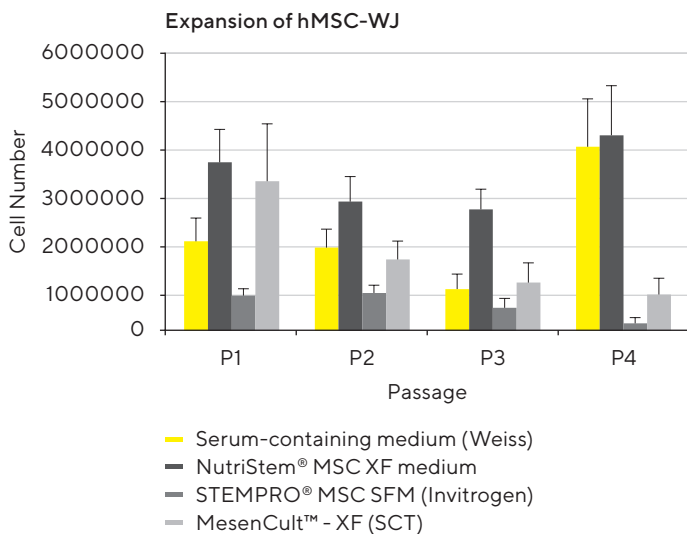
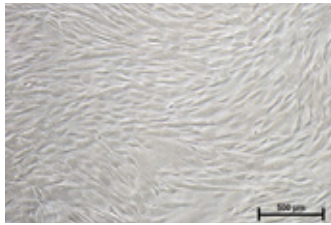
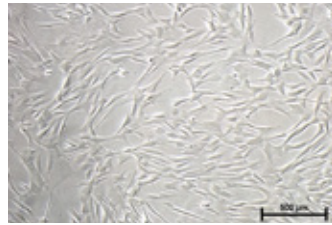


Figure 1: NutriStem® MSC XF Medium promotes superior proliferation and expansion of hMSCs over time as compared to other serum-free and serum-containing media.



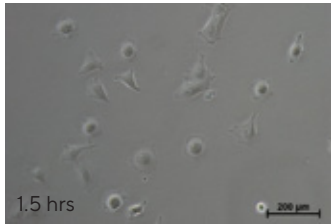
Recombinant Trypsin Solution



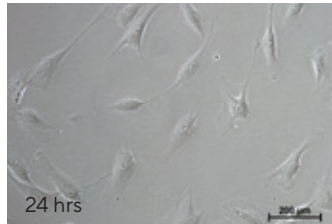
Crude Trypsin-EDTA Solution

Figure 2: MSC Dissociation Solutions.

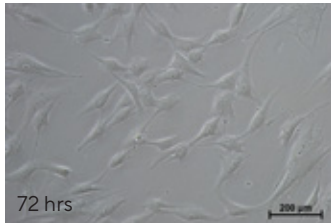
Recovery of BM-hMSC after dissociation with either Recombinant Trypsin Solution or Recombinant Trypsin-EDTA Solution and re-seeding on plates pre-coated with the MSC Attachment Solution and cultured in MSC NutriStem® XF Medium. Images were taken on Day 5 post-dissociation (100X).



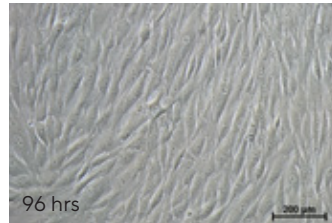
1.5 hrs



24 hrs



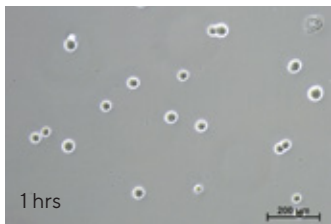
72 hrs



96 hrs

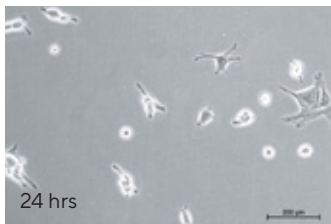
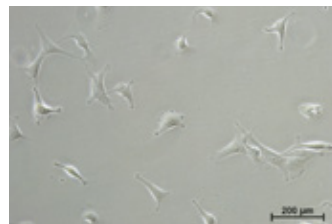
Figure 3: NutriFreez® D10 Cryopreservation Medium. Images show the recovery of BM-hMSC after thawing. Cells were frozen using NutriFreez® D10 Cryopreservation Medium, thawed, and re-seeded in MSC NutriStem® XF Medium on plates pre-coated with MSC Attachment Solution. Images were taken at the indicated time points post-thawing (200X).

A

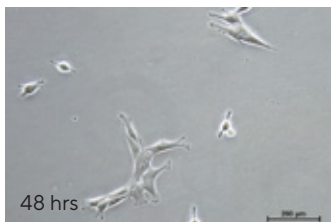
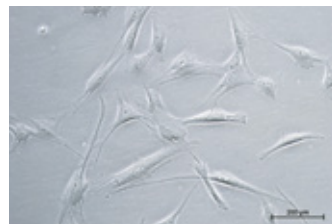


1 hrs

B



24 hrs



48 hrs

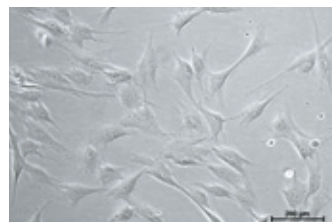


Figure 4: MSC Attachment Solutions. The use of MSC Attachment Solution greatly enhances BM-hMSC attachment and growth in culture. Cells in panel A images were cultured without MSC Attachment Solution. Cells in panel B were cultured with MSC Attachment Solution. Images were taken at the indicated time points post-seeding (200X).

Ordering Information

Cat. #	Product
05-200-1	MSC NutriStem® XF Basal Medium
05-201-1	MSC NutriStem® XF Supplement Mix
05-202-1	MSC NutriStem® XF Basal Medium, without Phenol Red
05-760-1	NutriCoat™ Attachment Solution
05-752-1	MSC Attachment Solution
05-713-1	NutriFreez® D10 Cryopreservation Medium
03-078-1	Recombinant Trypsin Solution
03-079-1	Recombinant Trypsin-EDTA Solution
PLTGOLD100R	PLTGold® Human Platelet Lysate (Research-grade)
PLTGOLD100GMP	PLTGold® Human Platelet Lysate (Clinical-grade)

Also available

MSCgo™ Differentiation Media

A unique line of complete, serum-free, and xeno-free media for efficient and reproducible differentiation of hMSCs.

- MSCgo™ Osteogenic XF Medium
- MSCgo™ Rapid Osteogenic XF Medium
- MSCgo™ Chondrogenic XF Kit
- MSCgo™ Adipogenic XF Kit

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Phone: 972 4 9960595

 For further contacts, visit
www.sartorius.com

Specifications subject to change without notice.

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StemPro™ MSC SFM

Catalog Number A10332-01

Pub. No. MAN0007328 Rev. 2.0



WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](https://www.thermofisher.com/support).

Product description

StemPro™ MSC SFM is a serum-free medium specially formulated for the growth and expansion of human mesenchymal stem cells (MSC) or adipose-derived stem cells (ADSC). Using StemPro™ MSC SFM, human MSC or ADSC can be expanded for multiple passages while maintaining their tri-lineage mesoderm potential (i.e., ability to differentiate into osteogenic, chondrogenic and adipogenic lineages). Each container is sterile filtered.

Contents and storage

Contents	Amount	Storage	Shelf life ^[1]
StemPro™ MSC SFM Basal Medium	1 × 500 mL	2°C to 8°C. Protect from light.	24 months
StemPro™ MSC SFM Auxiliary Concentrated Medium	1 × 75 mL	–20°C to –5°C. Protect from light.	

^[1] Shelf-Life duration is determined from Date of Manufacture.

Culture conditions

Media: Complete StemPro™ MSC SFM

Cell line(s): Human mesenchymal stem cells (MSC) or adipose-derived stem cells (ADSC)

Culture type: Adherent

Culture vessels: CELLStart™ Substrate coated T-Flasks.

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 4–6% CO₂ in air. Ensure proper gas exchange and minimize exposure of cultures to light.

Note: Procedures detailed in the following sections are for cultures in T-75 culture flasks (75 cm²). Volumes should be adjusted accordingly for desired vessel size.

Procedural guidelines

- Thaw StemPro™ MSC SFM Auxiliary Concentrated Medium overnight at 2°C to 8°C. Use thawed material immediately or aliquot (i.e., 15 mL) unused material and store at –20°C to –5°C protected from light. Avoid additional freeze-thaw cycles.
- Complete StemPro™ MSC SFM medium (basal medium, auxiliary concentrated medium, and L-glutamine or CTS™ GlutaMAX™-I Supplement) is stable for 4 weeks when stored in the dark at 2°C to 8°C, within the expiration date of all three components.

Prepare complete medium

StemPro™ MSC SFM Basal Medium requires supplementation with StemPro™ MSC SFM Auxiliary Concentrated Medium and L-glutamine or CTS™ GlutaMAX™-I Supplement. For 100 mL complete medium:

- Aseptically add 15 mL of StemPro™ MSC SFM Auxiliary Concentrated Medium to 84 mL of StemPro™ MSC SFM Basal Medium.
- Aseptically add 2 mM L-glutamine or CTS™ GlutaMAX™-I Supplement to the complete medium before use.
- Antibiotics can be used if desired, we recommend Gentamicin Reagent Solution (5 µg/mL final concentration).

General recommendations subculture MSC

- StemPro™ MSC SFM has been developed for the primary isolation and multi-passage expansion of human bone marrow-derived MSC and ADSC at greater than clonal densities, with optimal cell expansion observed at $\geq 5 \times 10^3$ cells/cm². (see Subculture in StemPro™ MSC SFM, step 11).
- Reduced seeding densities may result in suboptimal cell expansion. Optimal growth conditions must be determined for each application.

- It is recommended that human MSC in StemPro™ MSC SFM be subcultured when cell confluency reaches 60–80%, cells are in mid-logarithmic phase of growth and cell viability is at least 90%. Initiating cultures under suboptimal conditions may affect product performance. Transitioning MSC or ADSC from serum-containing medium to complete StemPro™ MSC SFM does not require an adaptation protocol.
- For optimal performance and cell growth, cultures should be re-fed every 2 days with fresh complete StemPro™ MSC SFM.

Coat culture flasks with CELLStart™ Substrate

1. Dilute CELLStart™ Substrate 1:100 in CTS™ DPBS with calcium, magnesium (i.e., 100 µL CELLStart™ Substrate into 10 mL of CTS™ DPBS with calcium, magnesium). Mix by gentle pipetting, do not vortex. Add 10 mL of the CELLStart™ Substrate to each flask, ensure complete surface coverage.

Note: Do not store diluted CELLStart™ Substrate solution; prepare fresh before each use.

2. Incubate at 37°C in a humidified atmosphere of 5% CO₂ in air for 60 minutes.
3. After incubation, remove flasks from the incubator and temporarily place them in a laminar flow hood until use. Immediately before use, remove all CELLStart™ Substrate and replace with complete medium.

Subculture in StemPro™ MSC SFM

1. Observe stock culture flask (cells growing in current medium formulation or in StemPro™ MSC SFM) with an inverted microscope and confirm that the cells are ready to be subcultured (60–80% confluent).
2. Pre-warm CTS™ TrypLE™ Select Enzyme and Complete StemPro™ MSC SFM to 37°C before use.
3. Aspirate spent medium from the flask and discard.
4. Wash cell monolayer with 5 mL pre-warmed CTS™ DPBS without calcium chloride, without magnesium chloride, aspirate and discard.

5. Add 3–5 mL CTS™ TrypLE™ Select Enzyme to each flask, ensure complete coverage of cell monolayer.

6. Incubate for 5–10 minutes at 37°C.

Note: Cells coming out of serum-containing medium may require a longer incubation time.

7. Check flasks with an inverted microscope for cell detachment. Firmly tap the flask as necessary to facilitate complete cell detachment.
8. Upon cell detachment, add 5 mL pre-warmed CTS™ DPBS with calcium, magnesium to each flask to completely cover the surface area.
9. Transfer cell suspension to a sterile 15-mL conical tube. Tap flask firmly, re-wash with 5 mL pre-warmed CTS™ DPBS with calcium, magnesium and collect.
10. Centrifuge tubes at 100–200 × g for 5 minutes at room temperature. Aspirate and discard DPBS being careful not to disturb cell pellet.
11. Resuspend cell pellet in a minimal volume of pre-warmed complete StemPro™ MSC SFM for cell counting, using a preferred counting method (e.g., Countess™ II Automated Cell Counter).
12. Remove CELLStart™ Substrate coating solution from each coated flask, add 15 mL pre-warmed complete StemPro™ MSC SFM.
13. Add 5×10^3 viable cells/cm² to each flask (i.e., 3.75×10^5 viable cells/T-75 flask).
Gently swirl cell suspension to ensure even distribution.
14. Incubate at 37°C in a humidified atmosphere of 5% CO₂.
15. Replace spent culture medium every 2–3 days with fresh, pre-warmed complete StemPro™ MSC SFM for optimal performance and cell growth.

Related products

Item	Source
CELLStart™ Substrate	A10142
CTS™ DPBS without calcium chloride, without magnesium chloride	A12856
CTS™ DPBS with calcium, magnesium	A12858
Gentamicin Reagent Solution (10mg/mL) liquid	15710
L-Glutamine (200 mM)	A29168
CTS™ GlutaMAX™-I Supplement	A1286001
CTS™ TrypLE™ Select Enzyme (1X), no phenol red	A12859
CTS™ Synth-a-Freeze	A13713
StemPro™ MSC SFM XenoFree	A10675-01
StemPro™ Adipogenesis Differentiation Kit	A10070
StemPro™ Chondrogenesis Differentiation Kit	A10071
StemPro™ Osteogenesis Differentiation Kit	A10072
StemPro™ Human Adipose-Derived Stem Cells	R7788
StemPro™ BM Mesenchymal Stem Cells, 1 × 10 ⁶ cells	A15652
Fetal Bovine Serum, MSC-Qualified	12662
Trypan Blue Stain	15250
Countess™ II Automated Cell Counter	AMQAX1000

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Contents

1. Description

1.1 Background information

1.2 Applications

2. Protocol

1. Description

This product is for research use only.

Components	500 mL StemMACS MSC Expansion Medium XF, human 7 mL StemMACS MSC Expansion Medium Supplement XF, human
Specifications	Osmolality: 270–320 mOsmol/kg pH: 7.0–7.6
Quality control	Functionality assay: Satisfactory expansion of MSCs derived from human bone marrow.
Storage	Store StemMACS MSC Expansion Medium XF, human protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label. Upon arrival store StemMACS MSC Expansion Medium Supplement XF, human at –20 °C. Aliquots of the supplemented complete medium should be stored at –20 °C. Avoid repeated freeze-thaw cycles. The expiration date is indicated on the vial label.

1.1 Background information

The StemMACS MSC Expansion Medium Kit XF, human is xeno-free. It is an optimized and standardized complete medium for the reproducible and reliable generation and expansion of mesenchymal stem cells (MSCs) from human bone marrow (BM) samples or other tissue sources. As MSCs appear at relatively low frequency in human BM samples and other tissues, their *in vitro* propagation is often necessary in order to obtain sufficient cell numbers for further experiments, such as *in vivo* transplantation studies in animals or *in vitro* differentiation or functional characterization. The formulation of StemMACS MSC Expansion Medium Kit XF was designed to efficiently support the expansion of MSCs *in vitro* while maintaining their differentiation potential. The medium formulation is xeno- and serum-free and is manufactured under strictly controlled conditions using ingredients of the highest quality. StemMACS Expansion Medium Kit XF offers consistent lot-to-lot performance and optimal conditions for the cultivation of MSCs.

1.2 Applications

- Expansion of MSCs from human bone marrow or other tissue sources.

2. Protocol

Before StemMACS MSC Expansion Medium Kit XF can be used in cell culture, the two kit components need to be mixed according to the following protocol to obtain the complete medium.

- Thaw StemMACS MSC Expansion Medium Supplement XF at 37 °C prior to use. Thawed material must be used immediately or aliquoted to the preferred sample volume, for example, 1.4 mL. Store aliquots at –20 °C. Avoid additional freeze-thaw cycles.
- To obtain the complete medium add 1.4 mL StemMACS MSC Expansion Medium Supplement XF to 100 mL StemMACS MSC Expansion Medium XF. The medium is ready-to-use now. Use the complete medium within 1 week when stored at 2–8 °C. For longer storage, prepare aliquots and store at –20 °C.

A detailed MSC cell culture protocol is available at www.miltenyibiotec.com/applications.

Refer to www.miltenyibiotec.com for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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CellCor™ CD MSC

Product Data Sheets



CellCor™ CD MSC

Human Mesenchymal Stem Cells



Ideal Culture Medium

It is important to select an appropriate medium since it can affect cell properties. CellCor™ CD MSC is the most ideal product to control culture conditions as it consists of chemically defined components.



Stable Maintenance

CellCor™ CD MSC shows superior proliferation until late passages and stable stemness (Tri-lineage differentiation and expression of MSC markers) in addition to low senescence and genetic stability.



Universal Use

CellCor™ CD MSC can be used for various tissue-derived MSCs (Adipose tissue, Bone marrow, Umbilical cord, and Wharton's jelly, etc.), and is ideal for exosome research.

Product Overview

Product (Formulation)	CellCor™ CD MSC Chemically Defined (Recombinants / Synthetics)
Catalog	YSP002
Application	Cell culture and expansion
Cell Type	Human Mesenchymal Stem Cells (MSCs)
Storage	Under -20°C (Expiry date on label)
Supplement	No supplement and coating reagent
Size	500 mL (Custom packaging available)
Area	For Research and Further Manufacturing Use



Why CellCor™ CD MSC?



Defined & Regulatory

Regulatory-friendly as it does not contain animal or human-derived components such as sera or hPL.

Stemness

Maintains stable stemness until late passages.
Genetic stability at the nuclear and chromosomal level in cell culture.

Consistency & Quality

Chemically defined products provide consistent products even in mass production.

Yield & Productivity

Excellent cell productivity with superior proliferation.

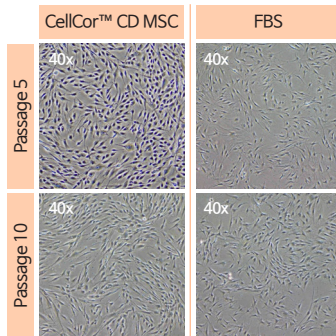
Reproducibility

Able to obtain highly reproducible experimental results.

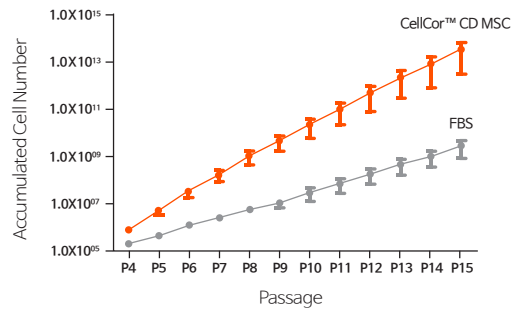
MSC Stemness

Superior & Standard Stem Cell Characterization

Cell Morphology

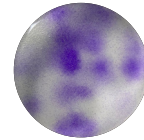


Proliferation

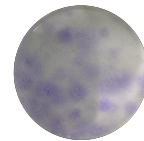


Colony Formation

(Passage 4)



CellCor™ CD MSC



FBS

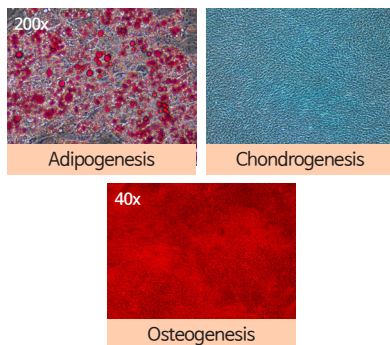
Figure 1. Superior Proliferation

AdMSCs cultured with CellCor™ CD MSC shows typical cell morphology, superior proliferation, and superior colony formation. (CellCor™ CD MSC : Chemically Defined Medium, FBS : FBS Containing Medium)

* Reference : Humana Press, 2008. 83-91

Tri-differentiation

(Passage 7)



MSC Marker

(Passage 7)

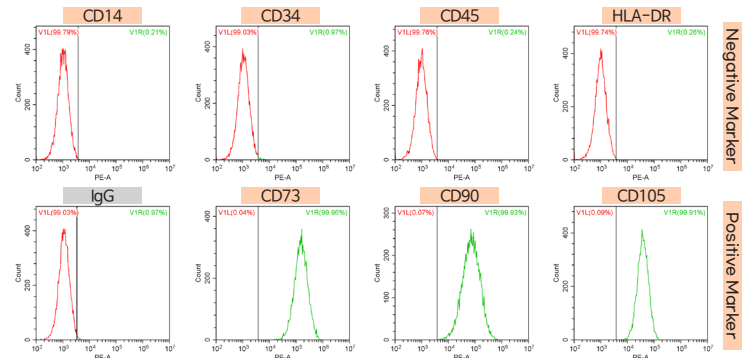


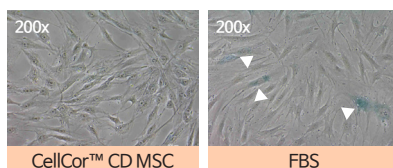
Figure 2. Stable MSC characterization

AdMSCs cultured with CellCor™ CD MSC maintains stemness : tri-lineage differentiation (adipogenesis, chondrogenesis, and osteogenesis) and MSC specific marker. (CD14, CD34, CD45, HLA-DR, CD73, CD90, and CD105) (Passage 7)

* Reference : Cytotherapy, 21 (10), 1019-1024

Senescence (Staining)

(Passage 5)



Senescence Marker(ODZ2)

(Passage 5)

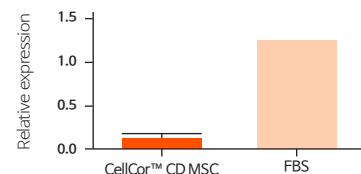


Figure 3. Low Senescence

AdMSCs cultured with CellCor™ CD MSC shows less b-galactosidase staining indicative of cellular senescence. (Passage 5) (CellCor™ CD MSC : Chemically Defined Medium, FBS: FBS Containing Medium)

* Reference : Cells, 2021 Jun; 10 (6) : 1301.

Genetic Stability

Minimize loss of genetic information

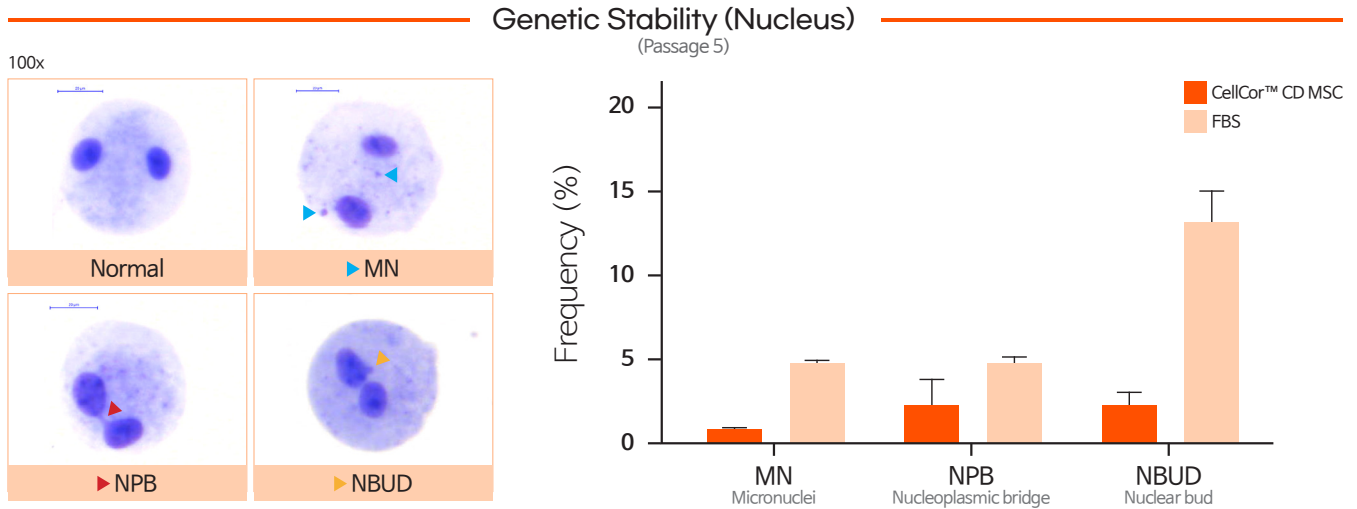


Figure 4. CBMN assay

AdMSCs cultured with CellCor™ CD MSC shows genetic stability at the nuclear level in cell culture. (Passage 6)
(CellCor™ CD MSC : Chemically Defined Medium, FBS: FBS Containing Medium)

* Reference : Cytotherapy, 21 (10), 1019–1024.

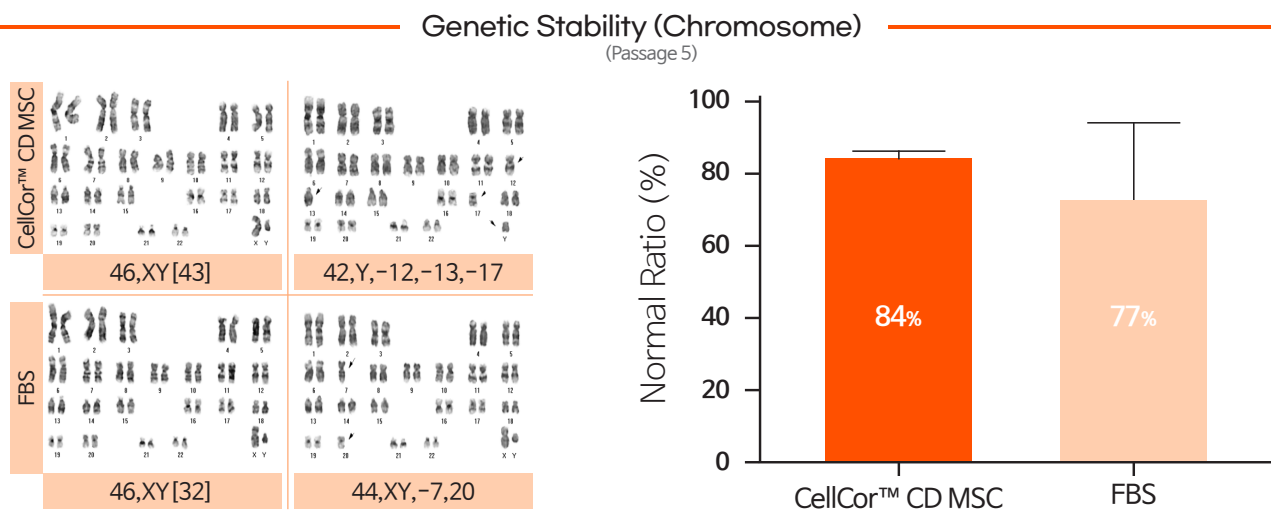


Figure 5. Karyotype analysis

AdMSCs cultured with CellCor™ CD MSC shows genetic stability at the chromosomal level in cell culture. (Passage 5)
(CellCor™ CD MSC : Chemically Defined Medium, FBS: FBS Containing Medium)

* Reference : Cytotherapy 18.3 (2016): 336–343.

Defined & Consistency

Unique features of Chemically Defined medium

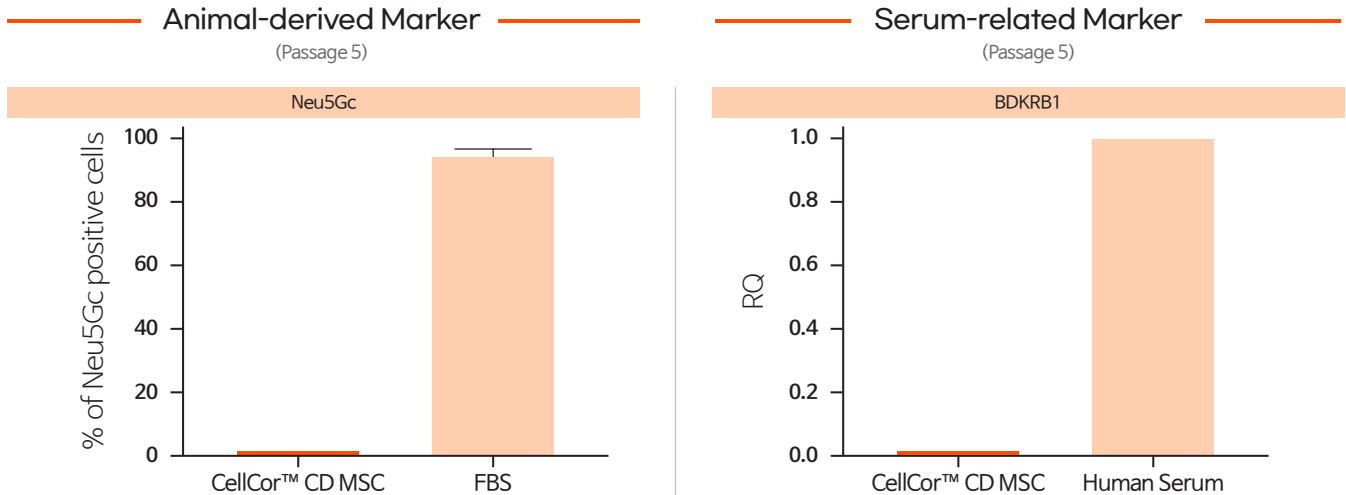


Figure 1. Chemically Defined Component

CellCor™ CD MSC is a chemically defined medium that shows it does not contain serum or animal-derived components. (Passage 5, Neu5Gc: Animal-derived marker, BDKRB1: Human-derived marker). (CellCor™ CD MSC: Chemically Defined Medium, FBS: FBS Containing Medium, Human Serum: Human Serum Containing Medium)

* Reference : Tissue Eng Part A, 2010. 16(4): p. 1143–55 / international journal of medical sciences 16.8 (2019): 1102.

Lot to Lot Variation

(Lot# N = 5)

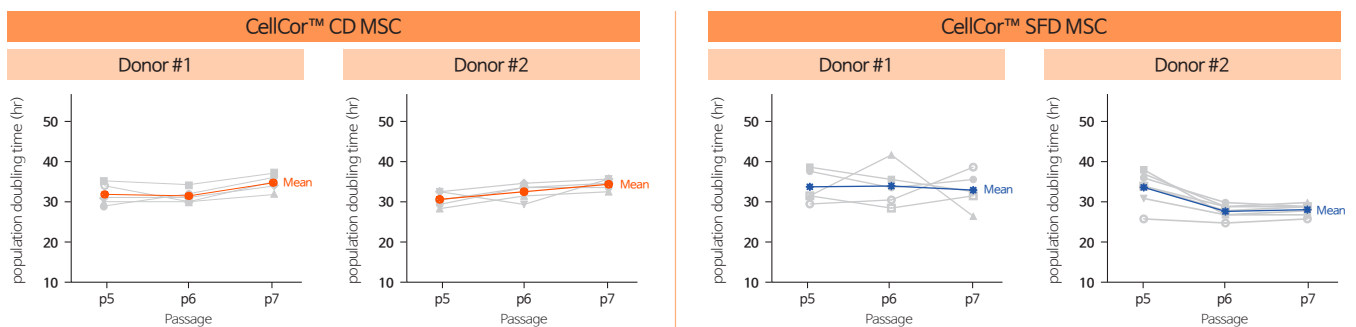


Figure 2. Consistency

CellCor™ CD MSC, Chemically defined medium, shows little variation as it does not contain animal- or human-derived components. It can improve the reproducibility of the experiment. (compared to serum-free medium; CellCor™ SFD MSC)

General & Universal

Applicable to various cell types

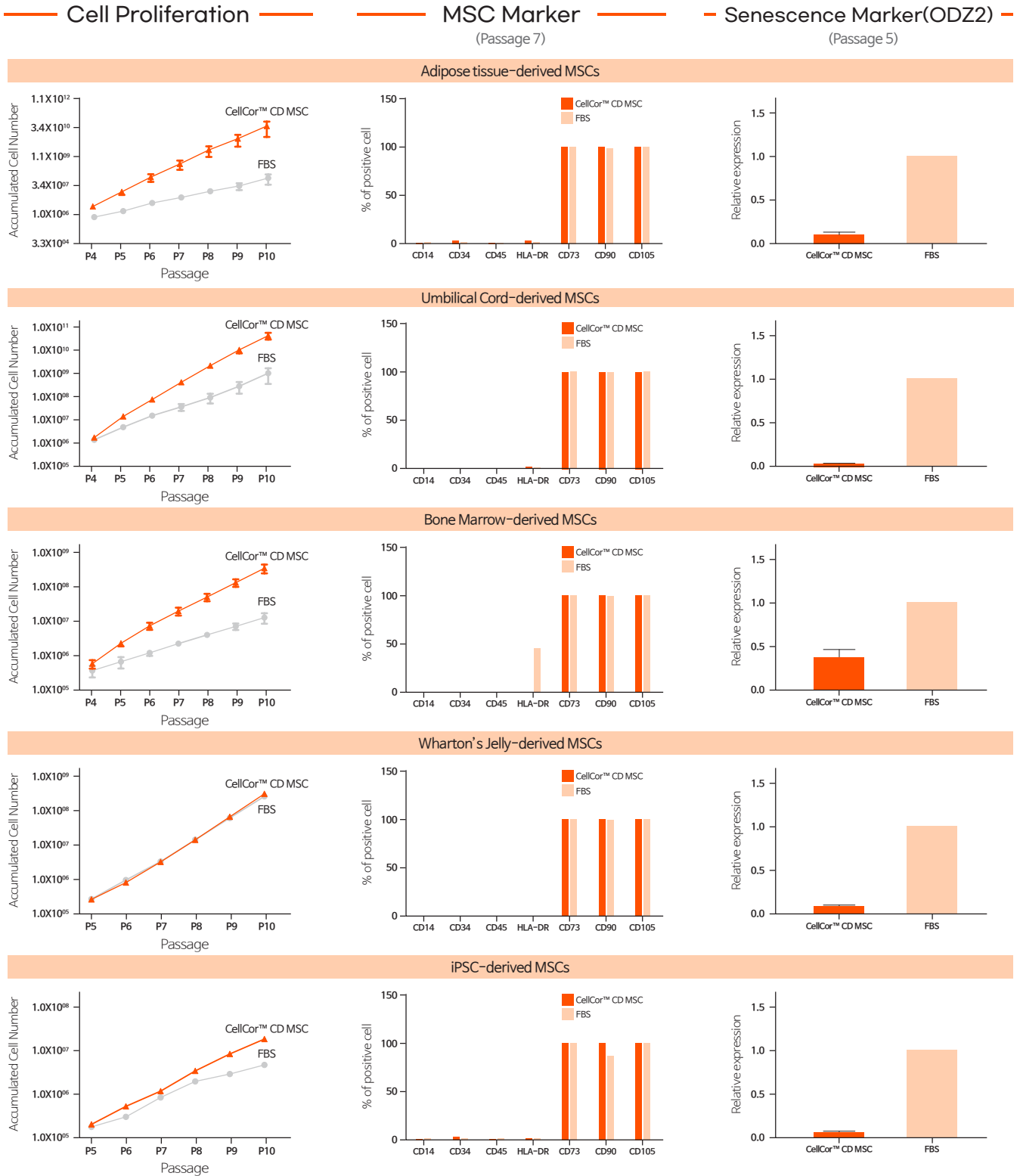
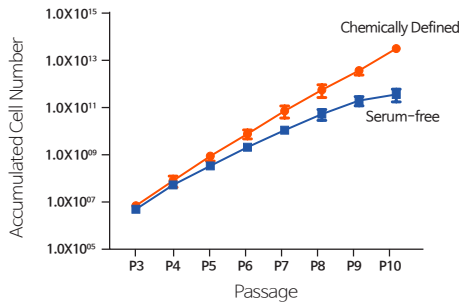


Figure 1. Characteristics of MSC from various tissue source

CellCor™ CD MSC could be cultured in various tissue- and cell-derived sources. (Adipose tissue, Umbilical cord, Bone marrow, Wharton's Jelly, iPSC) and shows to maintain stemness. (CellCor™ CD MSC : Chemically Defined Medium, FBS : FBS Containing Medium, Human Serum : Human Serum Containing Medium)

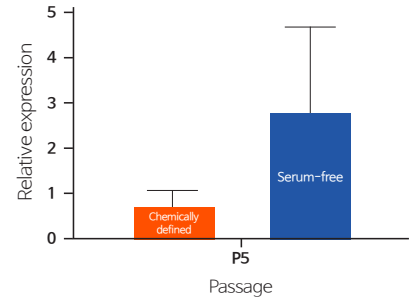
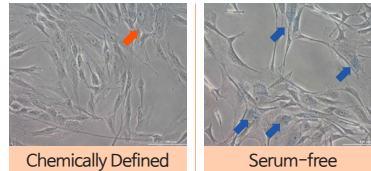
Chemically Defined vs Serum-free Media

Cell Proliferation



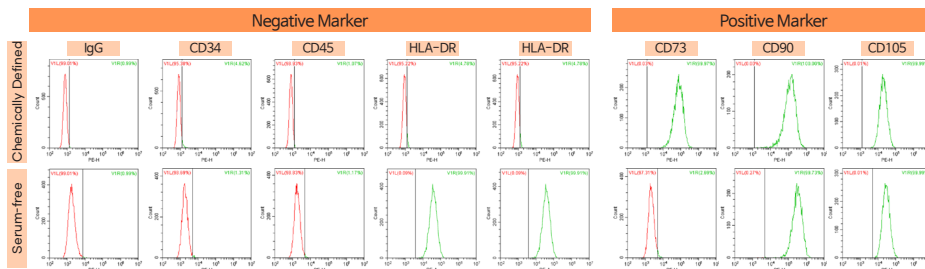
Senescence Staining

(Passage 5)



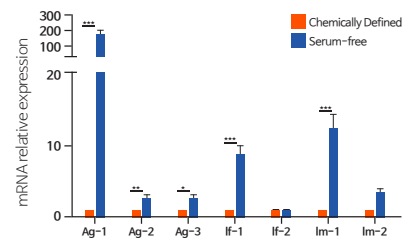
MSC Marker

(Passage 5)



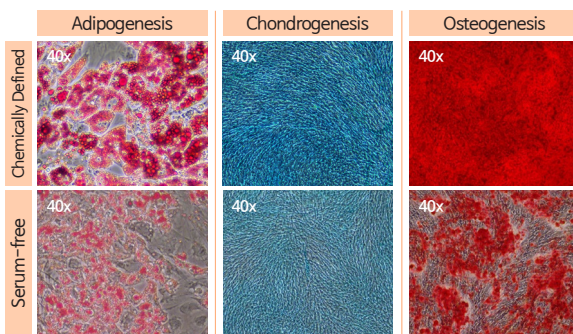
DEG

(Differentially Expressed Genes) (Passage 5)



Tri-differentiation

(Passage 5)



Genetic Stability

(Passage 5)

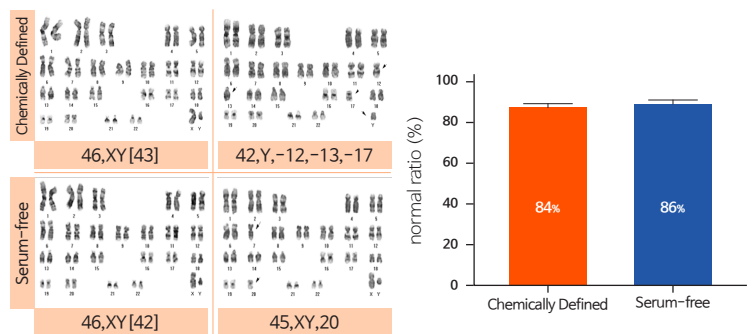


Figure 1. Compare MSC characterization to Serum-free Medium

Comparing with serum-free medium (Commercial product) AdMSCs cultured with CellCor CD MSC show superior cell proliferation, stable stemness, low senescence. Also, It shows low expression of immune- and inflammatory-related gene, and cell culture of genetic stability. (Passage 5, Ag-1 : IGF1P5, Ag-2 : Endoglin, Ag-3 : SOD2, If-1 : TNFRSF11B, If-2 : CSF1, Im-1 : CXCL12, Im-2 : PTX3)

CellCor™ CD MSC Applications





CellCor™ is a chemically defined medium specifically for cell culture.
With CellCor™ CD MSC as a start, we are constantly developing ways to expand
our product line to various cell specific chemically defined medium.

Technical Support

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Website Resources

Material Safety Data Sheets (MSDS)
Certificate of Analysis (CoA)
Product Information Sheets (PIS)
Product Manual Video
FAQs

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Product Information: StemFit For Mesenchymal Stem Cell

1. Introduction

StemFit For Mesenchymal Stem Cell (StemFit For MSC) is a chemically defined medium for mesenchymal stem cell (MSC) culture. This medium does not contain any undefined components such as serum or human platelet lysate, and it enables the maintenance of human bone marrow-derived MSC (BM-MSC), umbilical cord-derived MSC (UC-MSC) and adipose-derived stem cells (ADSC), under chemically defined conditions.

2. Materials Provided

Volume	Storage
500 ml	Store at -20 °C

Note: Add scaffold protein (e.g. iMatrix-511, Vitronectin)



3. Media Preparation

Use sterile techniques to prepare StemFit For MSC medium.

- 1) Thaw StemFit medium at room temperature (15-25 °C) or at 2-8°C overnight. Mix thoroughly.

CAUTION: Do not thaw StemFit For MSC at 37 °C, as it accelerates medium degradation

> Thawed StemFit For MSC medium may be stored at 2-8 °C for up to a month. **Protect from light.**

> Optionally, the medium can be stored as aliquots at -20°C until the expiration date. Do not re-freeze thawed aliquots.

- 2) Warm medium to room temperature and use immediately.

Key points for successful culture



Do not thaw the media at 37°C because it accelerates degradation of media.



Add iMatrix-511 or use matrix coated plate.



Remove dissociation reagent completely after centrifugation.



Avoid over confluent culture because that makes it difficult to detach and collect cells.

4. Thawing Protocol of Cryopreserved MSC

A) Use of Non-Coated Vessels

1. Prepare "StemFit For MSC + iMx medium" by adding iMatrix-511 (0.5 mg/mL) to StemFit For MSC medium to a final concentration of 0.2 µg/mL.
> **Example:** Add 4 µL of 0.5 mg/mL iMatrix-511 into 10 mL StemFit For MSC.
2. Add 9 mL of "StemFit For MSC + iMx medium" prepared in step 1 into a conical tube.
3. Quickly thaw the cryopreserved MSC cryovial in a 37 °C water bath within 2 min. Stop warming when the last piece of ice remains.
4. Transfer the cell suspension from the cryovial into the conical tube prepared in step 2.
5. Centrifuge at 500 x g for 5 min at room temperature. Decelerate without the use of an applied brake.
6. Aspirate the supernatant. Tap the tube to loosen the pellet and resuspend the cells with "StemFit For MSC + iMx medium".
7. Determine the cell concentration.
8. Seed cells at 5,000-10,000 cells / cm² in "StemFit For MSC + iMx medium".
> **Example:** 5.0 x 10⁴ cells / 2 mL / well in 6 well plate.
> **Example:** 3.8 x 10⁵ cells / 10-15 mL in T75 flask.
9. Continue to C)

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B) When Using ECM-Coated Culture Vessels

iMatrix-511, Fibronectin, or Vitronectin-coated plates are also compatible for use with StemFit For MSC.

1. Add 9 mL of StemFit For MSC into a polypropylene (PP) conical tube.
2. Quickly thaw the cryovial in a 37 °C water bath within 2 min. Stop warming when the last piece of ice remains.
3. Transfer cell suspension from cryovial into the conical tube prepared in step 1.
4. Centrifuge at 500 x g for 5 min at room temperature. Decelerate without the use of an applied brake
5. Aspirate the supernatant. Tap the tube to loosen the pellet and resuspend the cells with StemFit For MSC medium.
6. Determine the cell concentration.
7. Seed cells at 5,000-10,000 cells / cm² in StemFit For MSC medium.
 > **Example:** 5.0 x 10⁴ cells / 2 mL / well in 6 well plate. > **Example:** 3.8 x 10⁵ cells / 10-15 mL in T75 flask.
8. Continue to C)

C) Culture for both A) and B) (Culture for Both A and B)

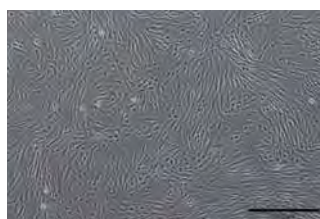
1. Culture cells at 37°C, 5% CO₂
2. Change the medium once in 2-3 days.

Note: iMatrix-511 is not required except for re-plating cells after passage.

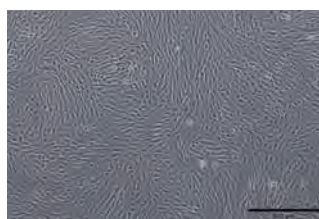
3. Subculture when cells are approximately 70-90% confluent.

Caution: Do not allow cells to become over confluent as shown in (c), since it will be difficult to detach and collect cells.

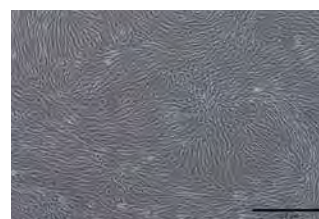
Passage should be done while cells are between conditions shown in (a) and (b).



(a) 70% confluent



(b) 90% confluent



(c) over confluent

5. Cell Expansion

1. Prepare "StemFit For MSC + iMx medium" by adding iMatrix-511 (0.5 mg/mL) to StemFit For MSC medium to a final concentration of 0.2 µg/mL.
 > **Example:** Add 4 µL of 0.5mg/mL iMatrix-511 into 10 mL StemFit For MSC.
Note: Users can use appropriate ECM-coated plate instead of adding iMatrix-511 into the medium. iMatrix-511, Fibronectin, or Vitronectin-coated plates are compatible for use with StemFit For MSC.
2. Aspirate the medium and wash on e with PBS.
3. Add Cell Detachment Solution [e.g. TrypLE™ Select (Thermo Fisher) or Accumax (MERCK Millipore)]
 > **Example:** 500 µL / well for 6 well plate. > **Example:** 4 mL / flask for T75 flask.
4. Incubate at 37°C for 10 mins until all cells are rounded and dissociation of cells is apparent.
5. Pipette the cells in the Cell Detachment Solution to fully dissociate cells and transfer to a PP conical tube.
6. To collect cells remaining in the vessel, add "StemFit For MSC + iMx medium" to the well / flask and then transfer to the conical tube.
 > **Example:** 1 mL / well for 6 well plate. > **Example:** 8 mL / flask for T75 flask.
7. Centrifuge at 200 x g for 5 min at room temperature.
8. Aspirate the supernatant completely.
Caution: Eliminate dissociation reagent completely. Remaining dissociation reagent may inhibit cell attachment to culture vessel.
9. Tap the tube to loosen the pellet and resuspend the cells with 0.5-1 mL "StemFit For MSC + iMx medium".
Note: Please adjust the volume of medium according to the culture scale.
10. Determine the cell concentration.
11. Seed the cells at 5.0 x 10³ cells / cm² in "StemFit For MSC + iMx medium".
 > **Example:** 5.0 x 10⁴ cells / 2 mL / well in 6 well plate. > **Example:** 3.8 x 10⁵ cells / 10-15 mL in T75 flask.
Note: Users can use matrix-coated plate instead of adding iMatrix-511 into the media.
12. Culture the cells at 37°C, 5% CO₂.
13. Change the medium once in 2-3 days.
Note: iMatrix-511 is not required except for re-plating cells after passage.
14. Subculture when cells are approximately 70-90% confluent.

Caution: Do not allow cells to become over confluent since it will be difficult to detach and collect cells. Passage should be done while cells are between conditions shown in (a) and (b) of step 3 in chapter 4 part C (Thawing protocol of cryopreserved MSC).

6. Precaution and Disclaimer

StemFit For MSC is for research use only and is not intended for human or animal diagnostic or therapeutic uses.

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Version 2 (July, 2020)

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