TECHNICAL MANUAL

Culture of Human Mesenchymal Stem Cells Using MesenCult™-XF Medium

STEMCELL™ TECHNOLOGIES

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1.0 Materials

1.1 MesenCult™-XF Medium and Accessory Components

MesenCult™-XF Medium (Catalog #05420) is a standardized, xeno-free, serum-free medium for the culture of human mesenchymal stem cells (MSCs). MesenCult™-XF Medium is optimized for the expansion of bone marrow-derived human mesenchymal stem cells in vitro as well as their enumeration using the colony-forming unit - fibroblast (CFU-F) assay. MesenCult™-XF Medium supports long-term growth of MSCs and cells maintain multi-lineage differentiation potential.

MesenCult™-XF Medium must be used in conjunction with the MesenCult™-SF Attachment Substrate (Catalog #05424) and the MesenCult™-ACF Dissociation Kit (Catalog #05426); components are tested for optimal cell adherence when MSCs are cultured with MesenCult™-XF Medium.

1.2 Storage of MesenCult™-XF Medium and Accessory Components

Table 1. MesenCult™-XF Medium and Accessory Component Storage and Stability

Catalog #	Description	Unit Size	Storage and Stability Conditions
05420	MesenCult™-XF Medium		
05421	MesenCult™-XF Basal Medium	400 mL	Product stable at 2 - 8°C for one year from date of manufacture as indicated on label.*
05422	MesenCult™-XF Supplement (5X)	100 mL	Product stable at -20°C for one year from date of manufacture as indicated on label. Storage at 2 - 8°C is not recommended.* *Storage of 10 mL aliquots at -20°C is possible; do not freeze-thaw supplements more than twice.*
05424	MesenCult™-SF Attachment Substrate		
05424	MesenCult™-SF Attachment Substrate§	5 mg	Product stable at 2 - 8°C until expiry date indicated on label. Do not freeze. Following reconstitution, the solution should be stored at -20°C. It is recommended to aliquot the reconstituted solution into multiple vials of 100 - 500 µL. Avoid additional freeze-thaw cycles. *Gently mix or pipette MesenCult™-SF Attachment Substrate.*
05426	MesenCult™-ACF Dissociation Kit		
05427	MesenCult™-ACF Enzymatic Dissociation Solution	250 mL	Product stable at -20°C until expiry date as indicated on label. This product should be thawed at 2 - 8°C, mixed gently, and stored in small working volumes at -20°C. Once thawed, solution is stable at 2 - 8°C for 4 days.
05428	MesenCult™-ACF Enzyme Inhibition Solution	250 mL	Product stable at -20°C until expiry date as indicated on label. This product should be thawed at 2 - 8°C, mixed gently, and stored in small working volumes at -20°C. Once thawed, solution is stable at 2 - 8°C for 4 days.

***** After MesenCult™-XF Supplement (Catalog #05422) has been added to MesenCult™-XF Basal Medium (Catalog #05421) storage at 2 - 8°C is recommended for no more than 5 days.

§ MesenCult™-SF Attachment Substrate must be resuspended and diluted prior to use (refer to Section 2.1).

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1.3 Additional Required Reagents

- L-Glutamine (Catalog #07100)
- Dulbecco's PBS without Ca^{++} and Mg^{++} (Catalog #37350)
- Lymphoprep™ (Catalog #07801)*
- 3% Acetic Acid with Methylene Blue (Catalog #07060)
- Trypan Blue (Catalog #07050)
- Human Serum Albumin (HSA; quality cell culture-tested and verified non-toxic for MSCs, e.g. 10% HSA in Iscove's MDM (Catalog #09350))
- 0.5 M EDTA (Ethylenediaminetetraacetic acid)
- Sterile distilled water
- Minimum Essential Medium Eagle (MEM), Alpha Modification (Catalog #36453; optional)

To stain CFU-F, the following additional materials are required:

- Methanol, ACS Grade (BDH Catalog #ACS531)
- Giemsa Stain Solution (EMD Chemicals Catalog #R03055)

1.4 Required Equipment

- Biohazard Safety Cabinet certified for Level II handling of biological materials
- 37°C incubator with humidity and gas control to maintain >95% humidity and an atmosphere of 5% CO_2 in air (e.g. Forma 3326)
- Bench top centrifuge with swinging bucket rotor (e.g. Beckman TJ-6)
- Inverted microscope
- Standard light microscope (for cell counting)
- Hemacytometer
- Pipette-aid (e.g. Drummond Scientific)
- Sterile pipettes: 1 mL, 5 mL and 10 mL
- Micropipette with 20 µL, 200 µL and 1 mL sterile tips
- 14 mL polystyrene tubes (BD Catalog #352057)
- 50 mL conical tubes (BD Catalog # 352070)
- Parafilm® (Sigma Catalog #P7793)*
- Recommended tissue culture plates/flasks:

Size	Suggested Plates/Flasks
6-well plate	Corning Catalog #3516
T-25 cm^2	BD Falcon™ Catalog #353109
T-75 cm^2	BD Falcon™ Catalog #353136

* Lymphoprep™ is a trademark of Axis-Shield.
Parafilm® is a registered trademark of American Can Co.

2.0 Preparation of Reagents

2.1 Reconstitution of MesenCult™-SF Attachment Substrate

1. Dissolve the lyophilizate with 5 mL sterile tissue grade water to obtain a final concentration of 1 mg/mL. Do not agitate or pipette vigorously.
2. Incubate for 30 - 60 minutes at 37°C to ensure the lyophilizate is fully in solution. Swirl gently.
3. Following reconstitution, the solution should be stored at -20°C. It is recommended to aliquot the reconstituted solution into multiple vials of 100 - 500 µL. Avoid additional freeze-thaw cycles.

2.2 Coating Plates with MesenCult™-SF Attachment Substrate

1. Coat plates one day prior to usage (i.e: coat overnight at 2 - 8°C), or if time is limited, coat for 2 hours at room temperature (15 - 25°C). **It is recommended to coat plates overnight.**
2. Plates must be coated with different dilutions of MesenCult™-SF Attachment Substrate (Catalog #05424) when performing CFU-F assays and when expanding cells in culture.

   Coating plates for CFU-F assay:
   Dilute MesenCult™-SF Attachment Substrate to a **1 in 30 final dilution** in sterile PBS without Ca^{++} and Mg^{++} (Catalog #37350). Gently mix by inverting the tube twice. Prepare an amount slightly more than required to account for pipetting variability. For example, to coat one 6-well plate, dilute 160 µL MesenCult™-SF Attachment Substrate in 4.8 mL PBS.

   Add diluted MesenCult™-SF Attachment Substrate as follows:

Size	Volume of Attachment Substrate	Suggested Plates
6-well plate	800 µL/well	Corning Catalog #3516

   Coating plates for cell expansion:
   When culturing cells obtained from primary tissue: Dilute MesenCult™-SF Attachment Substrate to a **1 in 20 final dilution** in sterile PBS without Ca^{++} and Mg^{++} (Catalog #37350). Gently mix by inverting the tube twice. Prepare an amount slightly more than required to account for pipetting variability. For example, to coat one T-75cm^2 flask, dilute 250 µL MesenCult™-SF Attachment Substrate in 5 mL PBS.

   When culturing previously cultured cells: Dilute MesenCult™-SF Attachment Substrate to a **1 in 28 final dilution** in sterile PBS without Ca^{++} and Mg^{++} (Catalog #37350). Gently mix by inverting the tube twice. Prepare an amount slightly more than required to account for pipetting variability. For example, to coat one T-75cm^2 flask, dilute 185 µL MesenCult™-SF Attachment Substrate in 5 mL PBS.

   Add diluted MesenCult™-SF Attachment Substrate as follows:

Size	Volume of Attachment Substrate	Suggested Plates/Flasks
6-well plate	800 µL/well	Corning Catalog #3516
T-25cm^2 flask	2 mL/flask	BD Falcon Catalog #353109
T-75cm^2 flask	5 mL/flask	BD Falcon™ Catalog #353136

3. Wrap plates with Parafilm®, sealing the junction between the base and lid and incubate at 2 - 8°C (in the refrigerator) overnight or for 2 hours at room temperature (15 - 25°C). For flasks, seal the vent on the cap with Parafilm® and incubate as described.

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4. If plates/flasks were incubated overnight at 2 - 8°C, bring to room temperature (15 - 25°C) (approximately 20 minutes) prior to washing. Gently pipette off remaining MesenCult™-SF Attachment Substrate without touching the newly coated surface.
5. Wash plates/flasks once with sterile distilled water by slowly pipetting water down the side of the well/flask, being careful not to scrape the newly coated surface. Swirl gently to rinse the entire surface and then carefully aspirate off water.
6. Allow to dry for at least 15 minutes at room temperature (15 - 25°C) prior to use.

2.3 Preparation of Complete MesenCult™-XF Medium

1. Thaw MesenCult™-XF Supplement (5X; Catalog #05422) overnight at 2 - 8°C. MesenCult™-XF Supplement can be aliquoted into smaller working volumes and stored at -20°C until required for use. Repeated thawing and freezing is not recommended.
2. Add the entire MesenCult™-XF Supplement (100 mL) to one bottle (400 mL) of MesenCult™-XF Basal Medium (Catalog #05421).

   *Complete MesenCult™-XF Medium should be prepared in volumes that can be **used within 5 days**. Prepare an amount suitable for your needs by diluting MesenCult™-XF Supplement 1/5 (final dilution) in MesenCult™-XF Basal Medium (e.g. 20 mL MesenCult™-XF Supplement + 80 mL MesenCult™-XF Basal Medium).*
3. Add L-Glutamine (Catalog #07100) to a final concentration of 2 mM. This is now referred to as Complete MesenCult™-XF Medium.
4. Complete MesenCult™-XF Medium should be stored at 2 - 8°C for no more than 5 days.

3.0 Preparation of Human MSCs for Culture with MesenCult™-XF Medium

3.1 Preparation of Mononuclear Cell Suspension from Fresh Human Bone Marrow

When working with a fresh bone marrow (BM) sample, the cells need to be processed to remove red blood cells prior to culture.

1. Prepare 500 mL Isolation Buffer (PBS + 0.5% HSA + 2mM EDTA) by adding the following to 1X PBS:
   - 25 mL HSA[†] (10% stock solution)
   - 2 mL EDTA (0.5 M stock solution)
   - Make up to 500 mL with 1X PBS

   If any of the components are not sterile (i.e. EDTA), be sure to filter sterilize the individual components or the complete buffer with a 0.2 micron filter. Once made, the Isolation Buffer can be stored at 2 - 8°C.
2. Count the total number of nucleated cells in the BM sample by taking 10 µL BM and diluting it 1/50 - 1/100 with 3% Acetic Acid with Methylene Blue (Catalog #07060)[‡]. Count cells using a hemacytometer.
3. Warm 50 mL Isolation Buffer at room temperature for 20 minutes prior to use. Dilute bone marrow to a **5 in 14 final dilution** with room temperature (15 - 25°C) Isolation Buffer (e.g. dilute 25 mL BM with 45 mL Isolation Buffer for a total volume of 70 mL).
4. In three 50 mL conical tubes (BD Catalog # 352070), pipette 17 mL Lymphoprep™ (Catalog #07801) into each tube. Carefully layer 23 mL diluted BM on top of the Lymphoprep™ in each tube.
5. Centrifuge at room temperature (15 - 25°C) for 30 minutes at 300 x *g* in a bench top centrifuge with the **brake off**.
6. Remove and discard the upper plasma layer without disturbing the plasma:Lymphoprep™ interface. Carefully remove and retain the mononuclear cells located at the interface layer and place in a new 50 mL conical tube. Resuspend the mononuclear cells with 40 mL cold (2 - 8°C) Isolation Buffer. Mix gently by pipetting.
7. Centrifuge the cells at 300 x *g* for 10 minutes at room temperature (15 - 25°C) in a bench top centrifuge with the **brake on**. Remove the supernatant and resuspend cells in 1 - 2 mL cold Isolation Buffer.
8. Dilute cells 1/50 in 3% Acetic Acid with Methylene Blue[‡] and count the total number of nucleated cells using a hemacytometer.
9. Dilute cells in Complete MesenCult™-XF Medium at a final concentration of 1 x 10^6 cells/mL.

[†] 10% HSA in Iscove's MDM (Catalog #09350) has been tested and verified as non-toxic for MSCs and is suitable for use in the Isolation Buffer.

[‡] 3% Acetic Acid with Methylene Blue will lyse red blood cell and white blood cell membranes. The remaining white blood cell nuclei will stain lightly with Methylene Blue. Refer to the Product Information Sheet for 3% Acetic Acid with Methylene Blue (Catalog #07060) available at www.stemcell.com for more information on how to count cells using a hemacytometer.

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4.0 Colony Forming Unit - Fibroblast (CFU-F) Assay

4.1 Plating Cells for the CFU-F Assay

A fresh bone marrow sample or culture-expanded mesenchymal stem cells are required when performing CFU-F assays. Do not use previously frozen bone marrow mononuclear cells.

1. CFU-F assays must be performed using tissue culture-treated plates that have been coated with MesenCult™-SF Attachment Substrate (Catalog #05424) as described in Section 2.1
2. Using fresh bone marrow-derived mesenchymal stem cells processed according to Section 3.0, seed cells at three different densities (between 1.5 - 5 x 10^4 cells/cm^2) in Complete MesenCult™-XF Medium. Note: at this point you should have a stock solution of cells at 1 x 10^6 cells/mL.

Tissue Culture Vessel	Volume of Medium	Suggested Plating Densities	Volume of 1 x 10^6 cells/mL
6-well plate	2.5 mL/well	1.5 x 10^5 cells/well	150 µL
		2.5 x10^5 cells/well	250 µL
		5.0 x 10^5 cells/well	500 µL

OR

Using culture-expanded mesenchymal stem cells, seed between 25 - 250 cells per well of a 6-well plate at five different densities in Complete MesenCult™-XF Medium.

Plating different cell densities will ensure that the resulting numbers of colonies can be scored. The proliferative potential of CFU-F from various bone marrow samples is widely variable. If too few cells are plated, CFU-F may be undetectable or the number of colonies scored may be too low to give a reliable estimation of CFU-F. If too many cells are plated, too many CFU-F may grow such that individual colonies cannot be determined. Refer to Section 5.0 for representative images.

3. Place the cultures in a 37°C incubator with 5% CO_2 in air and 95% humidity for 9 - 12 days. After day 7, monitor the growth of colony-forming cells daily, to prevent overgrowth. Colonies are ready to be stained before colonies start touching the neighboring colonies.

   Note: Monitor CFU-F colony size. MSCs cultured in MesenCult™-XF Medium proliferate faster than cells cultured in a traditional serum-based medium and therefore CFU-F assays must be stopped earlier when cells are cultured in MesenCult™-XF Medium.

4.2 Giemsa Staining of CFU-F Colonies

1. Gently remove MesenCult™-XF Medium from CFU-F cultures and discard. Adherent CFU-F colonies will remain attached.
2. Gently wash colonies once with PBS (Catalog #37350) to remove any residual culture medium.
3. Fix cells by adding 2 mL Methanol, ACS Grade (BDH Catalog #ACS531) to each well of a 6-well plate. Incubate for 5 minutes at room temperature (15 - 25°C).
4. Remove methanol and discard. Let the culture dishes air dry at room temperature (~5 minutes).

5. Add 2 mL Giemsa Stain Solution (EMD Chemicals Catalog #R03055) to each well of a 6-well plate. Incubate 5 -10 minutes at room temperature.
6. Remove Giemsa Stain Solution and rinse with distilled water to remove unbound stain. Rinse until water remains clear.
7. Discard the distilled water and allow the tissue culture dishes to dry at room temperature with the lid open. *Note: 6-well plates typically take 2 - 3 hours to dry.*

4.3 Enumeration

1. Human bone marrow-derived CFU-F colonies are large enough to see with the naked eye following staining with Giemsa. We recommend taking a felt-tip pen and marking each CFU-F on the bottom of the well when counted. This prevents counting colonies more than once.

   It is important to note that some colonies do not take up enough stain to be easily visible macroscopically, and therefore it is important to verify the number of colonies counted by scoring colonies microscopically.

   Note: CFU-F cultured in MesenCult™-XF Medium have a slightly different morphology than CFU-F typically obtained when performing the CFU-F assay with the MesenCult™ Proliferation Kit (Human; Catalog #05411). Refer to Section 5.0 for images.
2. Ensure that there is a linear relationship between the cell numbers plated in a given well and the resulting colony numbers. For example, there should be twice as many CFU-F colonies when 5.0×10^5 cells are plated than when 2.5×10^5 cells are plated. It is important that the plates do not contain too many CFU-F such that individual CFU-F can not be determined, as this will lead to inaccurate estimations. Refer to Section 5.0 for images.
3. Each bone marrow sample is unique to that donor and the number of CFU-F may depend on a number of factors including age, presence of disease and previous treatments given to the patient.

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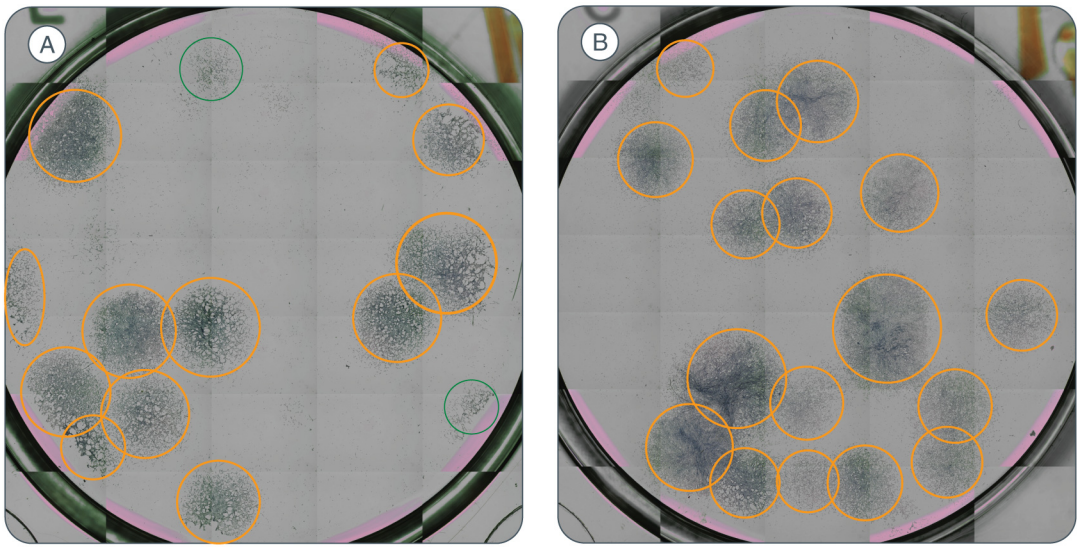
5.0 Representative Images of Human CFU-F

5.1 Enumeration: Scoring CFU-F Colonies

It is important that CFU-F colonies are distinct, so that an accurate assessment of CFU-F frequency can be determined. The images below show a good number of CFU-F for enumeration purposes.

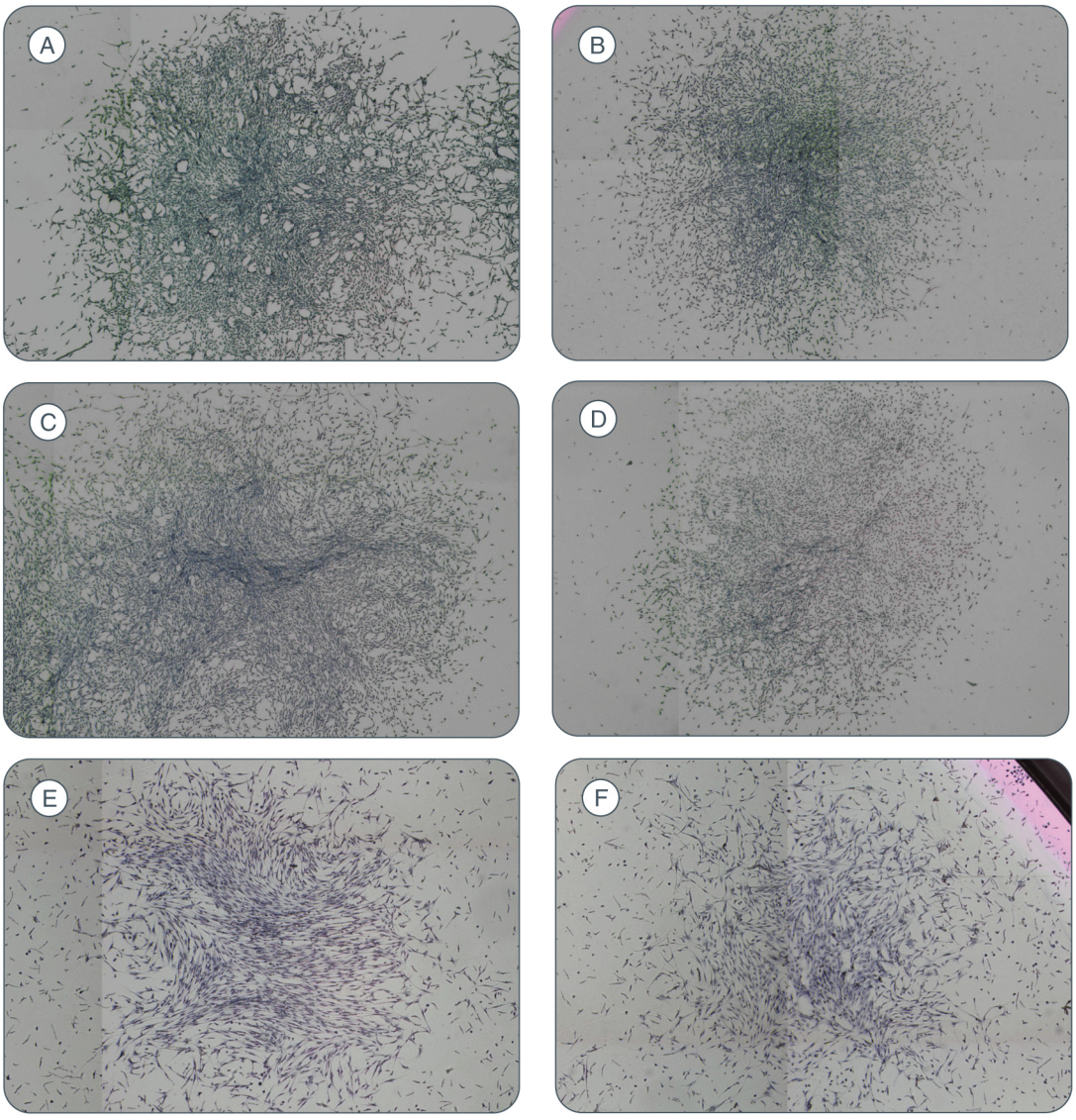
The colonies circled in orange are easily visible macroscopically. It is important to look at the CFU-F cultures under a microscope for confirmation because some colonies may not take up enough stain and could be missed when scored macroscopically (e.g. the colonies circled in green are only truly distinguishable as CFU-F under a microscope.



The CFU-F assay was performed in a 6-well plate (note the edges of the well in each image). A Lumenera Infinity2-3C camera was used to capture the images using Image Pro 6.2 software.

5.2 Comparison of CFU-F Colonies Cultured with MesenCult™-XF Medium or the MesenCult™ Proliferation Kit

The morphology of CFU-F colonies generated when MSCs are cultured in MesenCult™-XF Medium (A-D) differs from the morphology of CFU-F generated when MSCs are cultured with the MesenCult™ Proliferation Kit (Human; Catalog #05411) (E,F).



A Lumenera Infinity2-3C camera was used to capture the images using Image Pro 6.2 software.

6.0 Expansion of Cultured Mesenchymal Stem Cells

Note: The proliferation potential of cells obtained from different bone marrow donors is highly variable. To ensure that cultures contain an optimal number of cells for expansion, it is recommended to seed 2 - 3 different cell densities. If too few cells are plated, cells grow too slowly and reach recommended splitting density too late (cells start to detach from the surface). If too many cells are plated, the cells will reach confluence too fast and will become senescent and lose pluripotency.

6.1 Initial Plating of Mesenchymal Stem Cells for Expansion

1. When initially plating bone marrow mononuclear cells in MesenCult™-XF Medium for expansion, plate between 3.0 - 7.0 x 10^4 cells/cm^2 in Complete MesenCult™-XF Medium into tissue culture-treated plates/flasks that have been coated with MesenCult™-SF Attachment Substrate (Catalog #05424), as described in Section 2.1. If plating two cell densities, plate one flask at the lower end of the range and the other flask at the upper end of the range.

Tissue Culture Vessel	Volume of Medium	Surface Area	Suggested Plating Densities
6-well plate	2.5 mL/well	9.5 cm^2/well	3 x 10^5 cells/well
			4.5 x 10^5 cells/well
			6.0 x 10^5 cells/well
T-25cm^2	10 mL/flask	25 cm^2/flask	8.0 x 10^5 cells/flask
			10 x 10^5 cells/flask
			12.5 x 10^5 cells/flask
T-75cm^2	15 mL/flask	75 cm^2/flask	2.5 x 10^6 cells/flask
			4.0 x 10^6 cells/flask
			5.0 x 10^6 cells/flask

2. Place the cultures in a 37°C incubator with 5% CO_2 in air and 95% humidity for 9 - 13 days.[§]
3. Observe primary mesenchymal stem cells under a microscope 7 days post-plating to determine if they are ready for passaging or if the medium is acidic and a half-medium change needs to be performed.

   The cells are ready to be passaged when they reach 80% confluence. Normally cells reach 70 - 80% confluence between 9 - 13 days after initial plating of primary bone marrow mononuclear cells, but this depends on the donor and initial plating density. However, usually by day 10 - 11 the cells will be ready to passage. Refer to Section 7.2 for images of cells at different densities.

   Monitor the color of the medium after day 7: if the medium appears acidic (yellowish in color) prior to reaching 80% confluence, a half-medium change can be performed by removing 1/2 of the medium and replacing with fresh Complete MesenCult™-XF Medium warmed to 37°C.

[§] At this point, the cells are considered to be at Passage 0.

6.2 Passaging Cultured Mesenchymal Stem Cells

1. Warm the MesenCult™-ACF Enzymatic Dissociation Solution (Catalog #05427) and MesenCult™-ACF Enzyme Inhibition Solution (Catalog #05428) to room temperature (15 - 25°C). Do not incubate at 37°C.
2. To passage cells, slowly remove medium from cultures. The adherent cells will remain attached to the culture dish.
3. Wash cells with sterile PBS (Catalog #37350) to remove residual culture medium. Remove PBS.
4. Add 1 mL MesenCult™-ACF Enzymatic Dissociation Solution to each well of a 6-well plate, 3 mL to a T-25 cm^2 flask or 6 mL to a T-75cm^2 flask. Incubate at 37°C for 2 - 5 minutes.
5. After 2 minutes, observe cells under the microscope to ensure that all cells have detached. Gently tap plate/flask to detach remaining cells.
6. Add 1 mL MesenCult™-ACF Enzyme Inhibition Solution to each well of a 6-well plate, 3 mL to a T-25 cm^2 flask or 6 mL to a T-75cm^2 flask.
7. **6-well plate:**
   Collect cells into a 14 mL polystyrene tube and wash each well with 3 mL MEM Alpha (Catalog #36453)* to recover remaining cells. Add MEM Alpha* to bring the total volume to 8 mL.

   T-25cm^2:
   Collect cells into a 14 mL polystyrene tube and wash each flask with 5 mL MEM Alpha* to recover remaining cells. Add MEM Alpha* to bring the total volume to 12 mL.

   T-75cm^2:
   Collect cells into a 50 mL conical tube and wash each flask with 6 mL MEM Alpha* to recover remaining cells. Add MEM Alpha* to bring the total volume to 30 mL.
8. Centrifuge cells at 300 x *g* for 8 minutes at room temperature with the **brake on**.
9. Remove supernatant and resuspend cell pellet in 0.5 - 1 mL Complete MesenCult™-XF Medium.
10. Dilute cells with Trypan Blue (Catalog #07050)** and perform a viable cell count using a hemacytometer.

** Only non-viable cells will stain with Trypan Blue dye; viable cells remain unstained. Note: If cells are incubated for greater than 15 minutes in Trypan Blue, toxicity effects may occur and the viable cell count will be inaccurate. Refer to the Product Information Sheet for Trypan Blue (Catalog #07050) available at www.stemcell.com for more information on how to count cells using a hemacytometer.

* The cell washes can also be performed with Complete MesenCult™-XF Medium. It is important to add the additional medium when washing the cells, so that the MesenCult™-ACF Enzymatic Dissociation Solution is sufficiently washed from the cells.

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11. Plate cells in Complete MesenCult™-XF Medium into new tissue culture-treated plates/flasks that have been coated with MesenCult™-SF Attachment Substrate, as described in Section 2.1. The recommended plating density for passaged cells is between 1.5 - 4.0 x 10^3 cells/cm^2 (optimal plating densities for each tissue culture vessel are indicated in the table).

Tissue Culture Vessel	Volume of Medium	Surface Area	Suggested Plating Densities
6-well plate	2.5 mL/well	9.5 cm^2/well	1.5 x 10^4 cells/well
			3.0 x 10^4 cells/well
T-25cm^2	10 mL/flask	25 cm^2/flask	7.5 x 10^4 cells/flask
			12.5 x 10^4 cells/flask
T-75cm^2	15 mL/flask	75 cm^2/flask	15 x 10^4 cells/flask
			25 x 10^4 cells/flask

12. Place the cells in a 37°C incubator with 5% CO_2 in air and 95% humidity until they reach 80% confluence. When cells reach 80% confluence and are ready to be passaged, repeat steps 1 - 12 of Section 6.2. A half-medium change is only necessary if the medium appears acidic (yellowish in color) prior to reaching 80% confluence.

    *For culture-expanded mesenchymal stem cells (Passage 1 onward) it takes approximately **3 - 6 days** for the culture to reach 80% confluence (i.e. are ready for passaging), although this depends on the proliferative ability of cells from the particular bone marrow donor and the initial plating density.*

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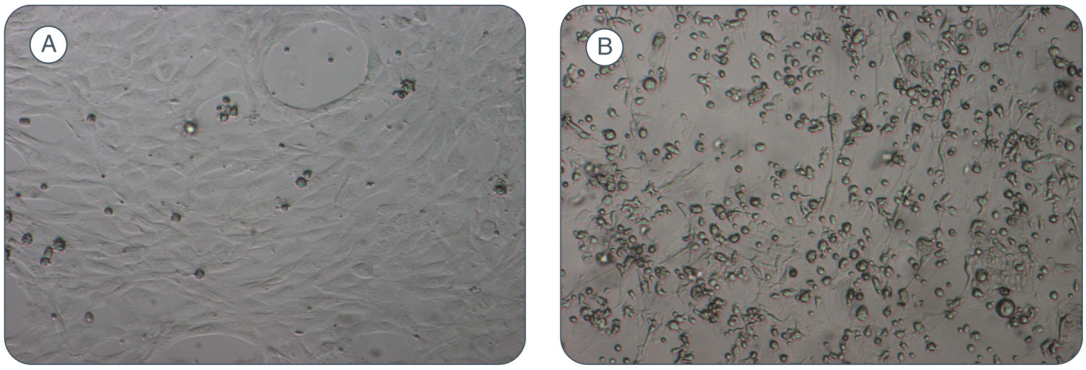
7.0 Representative Images of Human MSCs

7.1 Comparison of Primary Human Bone Marrow-Derived MSCs Cultured in MesenCult™-XF Medium or Traditional Serum-Based Medium

Passage 0 human bone marrow-derived mesenchymal stem cells show less hematopoietic cell contamination when cultured in MesenCult™-XF Medium (A) compared to serum-based medium (B).



Magnification: 5X

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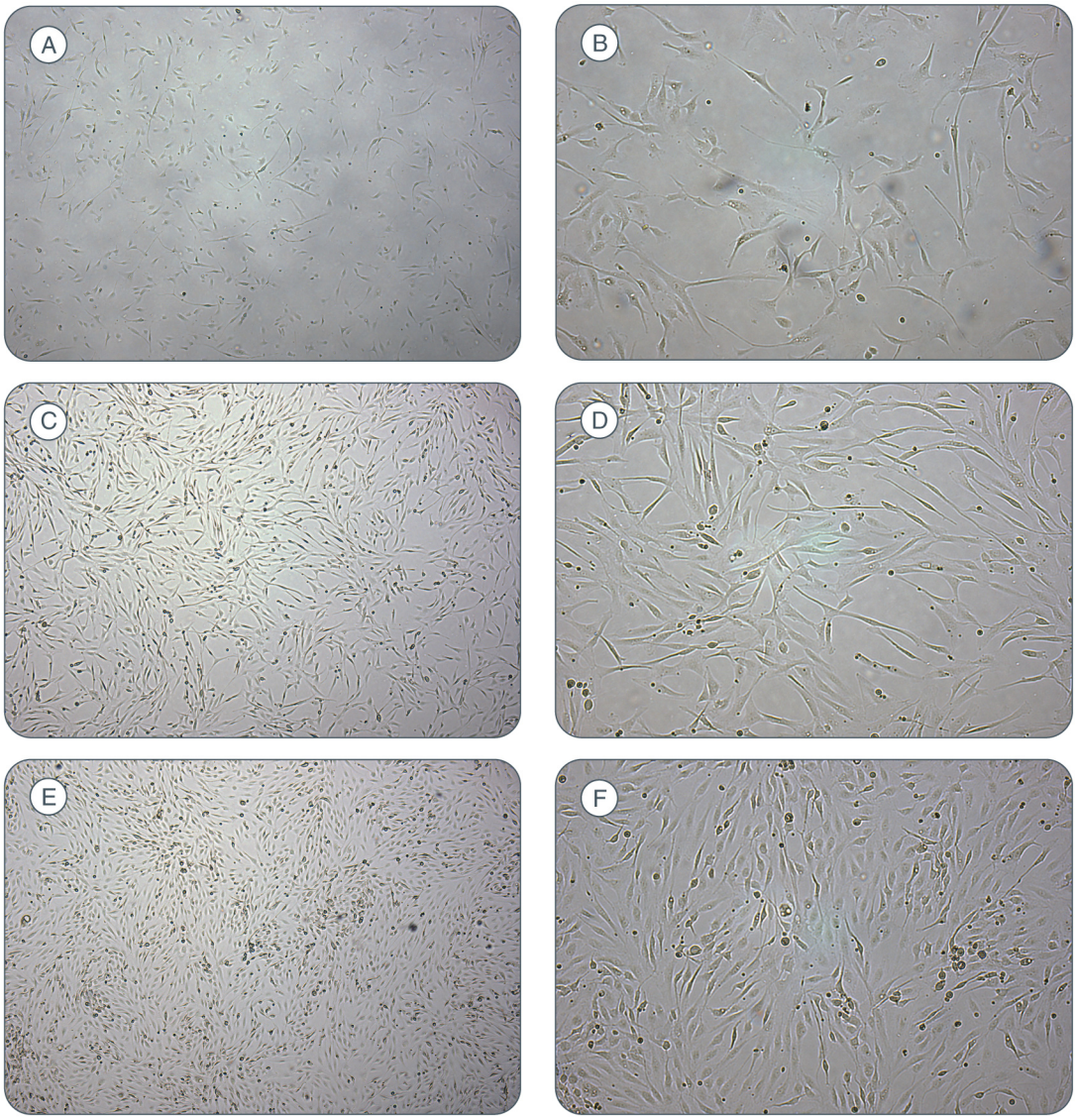
7.2 Cell Densities of MSCs Cultured in MesenCult™-XF Medium

It is important that cells are passaged when they reach 80% confluence. Figures C and D depict cells at an optimal density for passaging. Figures A and B are what cells should look like the day before passaging and in Figures E and F the cells too confluent (i.e. they should not be used).



Magnification: 5X (A, C, E); 10X (B, D, F).

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