MesenCult™-ACF Medium

Defined, animal component-free medium for human mesenchymal stem cells

Catalog #05440	500 mL
Catalog #05449	1 Kit

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

MesenCult™-ACF Medium is a standardized, animal component-free (ACF) and serum-free medium for the isolation and culture of human mesenchymal stem and progenitor cells (MSCs). MesenCult™-ACF 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 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 Medium must be used in conjunction with MesenCult™-ACF Attachment Substrate (Catalog #05444) and Animal Component-Free Cell Dissociation Kit (Catalog #05426), providing a complete, defined ACF culture system. Components of MesenCult™-ACF Attachment Substrate and Animal Component-Free Cell Dissociation Kit are prescreened and tested for optimal cell adherence when cells are cultured with MesenCult™-ACF 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. 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 Medium must be supplemented with L-Glutamine (Catalog #07100). See Preparation of Reagents and Materials.

Ordering Information

PRODUCT NAME	CATALOG #	SIZE	COMPONENTS
MesenCult™-ACF Medium*	05440	500 mL	• MesenCult™-ACF Basal Medium • MesenCult™-ACF 5X Supplement
MesenCult™-ACF Culture Kit	05449	1 Kit	• MesenCult™-ACF Basal Medium • MesenCult™-ACF 5X Supplement • MesenCult™-ACF Attachment Substrate

*Complete MesenCult™-ACF Medium must be supplemented with L-Glutamine (Catalog #07100).

Components

The following components are available as part of Catalog #05440 or #05449 and are not available for individual sale.

PRODUCT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
MesenCult™-ACF Basal Medium	05451	400 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.
MesenCult™-ACF 5X Supplement*	05452	100 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.
MesenCult™-ACF Attachment Substrate	05444	1 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.

*Each lot of MesenCult™-ACF 5X Supplement is used to prepare complete MesenCult™-ACF Medium and then performance tested in an expansion assay using human bone marrow MSCs.

None of the above components contain antibiotics.

Preparation of Reagents and Materials

Complete MesenCult™-ACF Medium

Use sterile techniques to prepare complete MesenCult™-ACF Medium (Basal Medium + 5X Supplement). The following example is for preparing 500 mL of complete medium. If preparing other volumes, adjust accordingly.

For aliquoting either the 5X Supplement or the complete medium, polypropylene tubes are strongly recommended (e.g. Fisher Catalog #05-539-5, 15 mL or Fisher Catalog #05-539-6, 50 mL); see Notes and Tips.

1. Thaw MesenCult™-ACF 5X Supplement at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.

   NOTE: Precipitate may be observed in thawed supplement or in complete medium in culture over time. This will not affect performance. If precipitate is observed in thawed supplement, leave at room temperature (15 - 25°C) for 30 minutes or incubate at 37°C for 15 minutes. Mix gently by inverting the supplement.

   NOTE: Once thawed, use immediately or aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing the aliquoted supplement, use immediately. Do not re-freeze.
2. Add 100 mL of MesenCult™-ACF 5X Supplement to 400 mL of MesenCult™-ACF Basal Medium. Mix thoroughly.
3. Add L-Glutamine (Catalog #07100) to reach a final concentration of 2 mM. Mix thoroughly.

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

MesenCult™-ACF Attachment Substrate-Coated Cultureware

Use sterile techniques when coating cultureware with MesenCult™-ACF Attachment Substrate (Catalog #05444).

NOTE: Use only tissue culture-treated cultureware.

1. Dilute MesenCult™-ACF Attachment Substrate in D-PBS (Without Ca++ and Mg++) (PBS; Catalog #37350) as follows:
   - For cultured cells, dilute Attachment Substrate1 in 300 in PBS. For example, add 20 µL of Attachment Substrate to 5.98 mL of PBS.
   - For primary bone marrow cells, dilute Attachment Substrate 1 in 150 in PBS. For example, add 40 µL of Substrate to 5.96 mL of PBS.
2. Gently mix the diluted MesenCult™-ACF Attachment Substrate. Do not vortex.
3. Immediately use diluted MesenCult™-ACF Attachment Substrate solution to coat cultureware. Refer to Table 1 for recommended coating volumes.

Table 1. Recommended Volumes for Coating Cultureware with MesenCult™-ACF Attachment Substrate

CULTUREWARE	VOLUME OF DILUTED MESENCULT™-ACF ATTACHMENT SUBSTRATE
6-well plate	1.0 mL/well
T-25 cm^2 flask	2.5 mL/flask
T-75 cm^2 flask	6.0 mL/flask

4. Gently spread MesenCult™-ACF Attachment Substrate solution evenly across the surface.
5. Incubate at room temperature (15 - 25°C) for at least 2 hours before use. Do not let the MesenCult™-ACF Attachment Substrate solution evaporate.

   NOTE: If not used immediately, cultureware must be sealed to prevent evaporation of MesenCult™-ACF Attachment 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 (15 - 25°C) for 30 minutes before proceeding to the next step.
6. Gently tilt the cultureware onto one side and allow excess MesenCult™-ACF Attachment 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.
7. Wash cultureware once using PBS (e.g. use 2 mL/well if using a 6-well plate).
8. Aspirate wash solution when MSCs are ready to be plated.

Directions for Use

Please read the entire protocol before proceeding.

Use sterile techniques when performing the following protocols.

A) Isolation of Human MSCs from Bone Marrow

B) Isolation of Human MSCs from Adipose Tissue

C) CFU-F Assay

D) 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™ (Catalog #07801) separation. If using other volumes, adjust accordingly.

1. Count nucleated cells in the BM sample using 3% Acetic Acid with Methylene Blue (Catalog #07060). Refer to the Product Information Sheet (Document #29604) for directions for use.
2. Split the BM sample into two 50 mL tubes (i.e. 12.5 mL of BM sample per tube).
3. Add 22.5 mL of room temperature (15 - 25°C) PBS containing 2 mM EDTA per tube.
4. Prepare three new 50 mL tubes and add 17 mL of Lymphoprep™ to each tube.
5. Layer 23 mL of the BM suspension (from step 3) on top of the Lymphoprep™ in each tube.
6. Centrifuge tubes at 300 x *g* for 30 minutes, with the **brake off**.
7. Collect the mononuclear cell (MNC) layer at the plasma:Lymphoprep™ interface and place in a single new 50 mL tube.

   NOTE: Sometimes it is difficult to see the cells at the interface. In this case, it is recommended to remove some of the Lymphoprep™ along with the enriched cells in order to maximize cell recovery.
8. Wash cells with cold (2 - 8°C) PBS containing 2 mM EDTA.
9. Centrifuge the tube at 300 x *g* for 10 minutes with the brake on.
10. Discard supernatant and resuspend the cell pellet in complete MesenCult™-ACF Medium (e.g. 2 - 4 mL of complete medium).
11. Count nucleated cells using 3% Acetic Acid with Methylene Blue.

B) Isolation of Human MSCs from Adipose Tissue

1. Add 2 - 4 mL of 0.25% Collagenase Type I (Catalog #07902) to the adipose tissue and finely mince tissue with a scalpel. Place it in a 50 mL tube.
2. Digest minced tissue in 5 mL of 0.25% Collagenase Type I per cm^3 of tissue at 37°C for 1 hour in a shaking water bath (e.g. for 3 cm^3 tissue use 15 mL collagenase).
3. Place the samples upright for 5 minutes to allow separation of the lipid layer from the aqueous layer.
4. Discard the top lipid layer with a pipette or aspirator.
5. Add PBS containing 2 mM EDTA to reach a final volume of 50 mL.
6. Centrifuge cells at 300 x *g* for 10 minutes, with the brake on.
7. Discard supernatant and resuspend the cell pellet in complete MesenCult™-ACF Medium.
8. Count nucleated cells using 3% Acetic Acid with Methylene Blue (Catalog #07060).

C) CFU-F Assay

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

NOTE: Only use tissue culture-treated cultureware.

1. Coat wells with MesenCult™-ACF Attachment Substrate (see Preparation of Reagents and Materials).
2. Plate cells in 2 mL of complete MesenCult™-ACF Medium per pre-coated well. Plate cells at three to four 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	CELLS PER CM^2	EXAMPLE OF CELL DENSITIES 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 - 10 x 10^3	0.5 x 10^4 1.5 x 10^4 3 x 10^4 8 x 10^4

3. Incubate cells at 37°C for 10 - 15 days until colonies (> 40 cells/colony) appear in the well.

   NOTE: If needed, perform a half-medium change on day 7 (i.e. aspirate 1 mL of medium and add 1 mL of complete MesenCult™-ACF Medium per well).

4. 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 cm^2 flask. If using other cultureware, adjust cell numbers and volumes accordingly.

NOTE: Only use tissue culture-treated cultureware.

1. Coat the flask with MesenCult™-ACF Attachment Substrate (see Preparation of Reagents and Materials).
2. Plate freshly isolated MSCs in 6 mL of complete MesenCult™-ACF Medium per pre-coated flask. Refer to Table 3 for recommended cell plating densities of freshly isolated primary cells.

Table 3: Recommended Cell Plating Densities for Cell Expansion of Freshly Isolated Cells

CELL TYPE	FRESHLY ISOLATED CELLS PER cm^2	EXAMPLE OF CELL DENSITIES PER T-25 cm^2 FLASK
BM-derived MSCs	4 - 10 x 10^4	1 - 2 x 10^6
Adipose-derived MSCs	3 - 20 x 10^3	0.8 - 5 x 10^5

3. Incubate cells 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 Medium per flask).

4. Passage cells using Animal Component-Free Cell Dissociation Kit (Catalog #05426). Use the following passaging protocol:
   i. Warm ACF Enzymatic Dissociation Solution (Component #05427) and ACF Enzyme Inhibition Solution (Component #05428) to room temperature (15 - 25°C). Do not incubate at 37°C.
   ii. Wash cells once with 2.5 mL of D-PBS (Without Ca++ and Mg++; Catalog #37350).
   iii. 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.
   iv. Add 2.5 mL of ACF Enzyme Inhibition Solution and collect cells in a polypropylene tube (e.g. 15 mL polypropylene tube [Fisher Catalog# 05-539-5]).
   v. Wash the flask with 5 mL of complete MesenCult™-ACF Medium and place into the same polypropylene tube as in step iv.
   vi. Centrifuge the tube at 300 x *g* for 8 minutes with the brake on.
   vii. Discard supernatant and resuspend the cell pellet in complete MesenCult™-ACF Medium (see Notes and Tips). Count and plate cells onto pre-coated cultureware according to Table 4.

Table 4: Recommended Cell Plating Densities for Expansion of Passaged Cells

CELL TYPE	PASSAGED CELLS PER cm^2	EXAMPLE OF CELL DENSITIES PER T-25 cm^2 FLASK
BM-derived MSCs	1.5 - 4 x 10^3	4 - 10 x 10^4
Adipose-derived MSCs	1.5 - 4 x 10^3	4 - 10 x 10^4

   viii. Incubate cells at 37°C until 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. Fisher Catalog #05-539-5, 15 mL or Fisher Catalog #05-539-6, 50 mL) during subculture will help to prevent the MSCs from sticking to the tubes.
- To break apart cell aggregates, use a 1 mL pipette to gently pipette the cell pellet up and down a few times [section D, step 4(vii)].