MesenCult™-ACF Plus Medium

Animal component-free medium for human mesenchymal stem cells

Catalog #05445 1 Kit
Catalog #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 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 12 months 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.

Preparation of Reagents and Materials

Complete MesenCult™-ACF Plus Medium

Use sterile techniques 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 polypropylene tubes (e.g. Sarstedt Catalog #72.785.005). Do not exceed the shelf life of the supplement. After thawing the aliquoted supplement, 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 (Catalog #07100) 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 techniques when coating cultureware with Animal Component-Free Cell Attachment Substrate (Component #07130).

NOTE: Use only tissue culture-treated cultureware.

1. Dilute Animal Component-Free Cell Attachment Substrate in D-PBS (Without Ca++ and Mg++) (PBS; Catalog #37350) as follows:
   - For cultured cells, dilute Attachment Substrate 1 in 300 in PBS. For example, add 20 µL of Substrate to 5.98 mL of PBS.
   - For human MSCs isolated from bone marrow, dilute Attachment Substrate 1 in 150 in PBS. For example, add 40 µL of Substrate to 5.96 mL of PBS.
   - For human MSCs isolated from adipose tissue, dilute Attachment Substrate 1 in 300 in PBS. For example, add 20 µL of Substrate to 5.98 mL of 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	1.0 mL/well
T-25 cm^2 flask	2.5 mL/flask
T-75 cm^2 flask	6.0 mL/flask

4. Gently tilt the cultureware to spread the 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 substrate solution evaporate.

   NOTE: If not used immediately, 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 (15 - 25°C) for 30 minutes before proceeding to the next 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.
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 2 x 50 mL conical tubes (e.g. Catalog #38010) (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. To each of 3 x 50 mL new conical tubes, add 17 mL of Lymphoprep™.
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 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.
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 Plus 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 in a 10 cm dish.

   NOTE: To maintain an ACF workflow, use ACF Collagenase Type I and II (Worthington Catalog #CLSAFA, Filtered), prepared at 0.2% in PBS containing 0.2% recombinant albumin.
2. Finely mince tissue with a scalpel. Transfer minced tissue to a 50 mL conical tube.
3. Add 5 mL of collagenase per cm^3 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 cm^3 of tissue.
4. Remove tube from the water bath or incubator. Place upright for 5 minutes to allow separation of the lipid layer from the aqueous layer.
5. Using a pipettor or aspirator, remove and discard the top lipid layer.
6. Add PBS containing 1 mM EDTA and 0.2% recombinant albumin (or 2% fetal bovine serum) to reach a final volume of 50 mL.
7. 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 (Catalog #07060).

C) CFU-F Assay

The following protocol is for setting up a CFU-F assay in a 6-well tissue culture-treated plate (e.g. Catalog #38016). 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 cm^2 flask. If using other cultureware, adjust cell numbers and volumes accordingly.

NOTE: Only use tissue culture-treated cultureware.

1. Coat a T-25 cm^2 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 10^4 freshly isolated cells/cm^2 (i.e. 1 - 2.5 x 10^6 cells per T-25 cm^2 flask)
   - For adipose-derived cells: 6 - 10 x 10^3 freshly isolated cells/cm^2 (i.e. 1.5 - 2.5 x 10^5 cells per T-25 cm^2 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 (Catalog #05426). Use the following passaging protocol:
   i. Coat a T-25 cm^2 flask with Animal Component-Free Cell Attachment Substrate (see Preparation of Reagents and Materials).
   ii. 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.
   iii. Wash cells once with 2.5 mL of 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 polypropylene tube (e.g. 15 mL conical tube [Catalog# 38009]).
   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 supernatant and resuspend the cell pellet in complete MesenCult™-ACF Plus Medium (see Notes and Tips). Count cells and seed a coated T-25 cm^2 flask (prepared in step i) at a density of 1.5 - 4 x 10^3 cells/cm^2 (i.e. 4 - 10 x 10^4 cells/flask).
   ix. 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. Catalog #38009 [15 mL] or Catalog #38010 [50 mL]) during subculture will help to prevent the MSCs from sticking to the tubes.
- 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)].