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 (AD-MSCs). 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 BM-MSCs and AD-MSCs, and enables cells to maintain robust multi-lineage differentiation potential in vitro.

Additionally, MesenCult™-ACF Plus Medium also supports the expansion of human embryonic stem (ES)- or induced pluoripotent stem (iPS)-derived mesenchymal progenitor cells (MPCs) and umbilical cord (UC)-derived MSCs, and is included in STEMdiff™ Mesenchymal Progenitor Kit (Catalog #05240) and MesenCult™-ACF Plus Umbilical Cord Culture Kit (Catalog #100-0234).

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 optimized cryopreservation in an ACF workflow, MesenCult™-ACF Freezing Medium (Catalog #05490) is recommended for cryopreservation of 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
3% Acetic Acid with Methylene Blue	07060
Animal Component-Free Cell Dissociation Kit • ACF Enzymatic Dissociation Solution • ACF Encyme Inhibition Solution	05426
Collagenase Type I (0.25%) OR Collagenase A, ACF	07902 OR 07434
D-PBS (Without Ca++ and Mg++)	37350
Hausser Scientific™ Bright-Line Hemocytometer	100-1181
L-Glutamine	07100
Lymphoprep™	07801
Non-treated culture dish,100 mm	e.g. 38045
Polypropylene conical tubes, 15 mL and 50 mL	e.g. 38009 and 38010
Tissue culture-treated 6-well plates	e.g. 38015
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 1 week**. 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 shown in Table 1.
2. Gently mix the diluted substrate solution. Do not vortex.
3. Immediately use the diluted substrate solution to coat cultureware. Refer to Table 2 for recommended coating volumes.
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, 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.
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 D-PBS (e.g. use 2 mL/well if using a 6-well plate).
8. Aspirate wash solution when MSCs are ready to be plated.

Table 1. Recommended Dilution Factors for Animal Component-Free Cell Attachment Substrate with D-PBS

CELL TYPE	DILUTION FACTOR
Isolation of human MSCs from primary tissue	
BM-MSCs	1 in 150
AD-MSCs	1 in 300
Expansion of cultured human MSCs or MPCs	
BM-MSCs	1 in 300
AD-MSCs	1 in 300
ES- or iPS-MPCs	1 in 300
UC-MSCs	1 in 150

For example, to prepare a 1 in 150 dilution, add 40 µL of ACF Attachment Substrate to 5.96 mL of D-PBS.

Table 2. 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

Directions for Use

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

A) Isolation of Human Mononuclear Cells from Bone Marrow

B) Isolation of Human MSCs from Adipose Tissue

C) CFU-F Assay

D) Expansion of Freshly Isolated Human MSCs

E) Expansion of Cultured Human MSCs

A) Isolation of Human Mononuclear Cells from Bone Marrow

The following protocol is for isolating mononuclear cells (MNCs) from 25 mL of fresh (< 24 hours after harvest) human BM using density gradient medium (i.e. Lymphoprep™) separation. If using other volumes, adjust accordingly.

1. Count nucleated cells in the BM sample using 3% Acetic Acid with Methylene Blue.
2. Split the BM sample into 2 x 50 mL conical tubes (i.e. 12.5 mL of BM sample per tube).
3. Add 22.5 mL of room temperature (15 - 25°C) D-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 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) D-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.
    The resulting cells are a mixture of MNCs; to purify and expand MSCs from this sample, proceed to section D.

B) Isolation of Human MSCs from Adipose Tissue

The following protocol is for isolating AD stromal vascular fraction (AD-SVF) cells from adipose tissue.

1. 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.
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 D-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.
   The resulting cells are a mixture of AD-SVF cells; to purify and expand MSCs from this sample, proceed to section D.

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 3 for recommended cell plating densities.

Table 3. 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
AD-MSCs	0.5 - 4 x 10^3	0.5 x 10^4 1 x 10^4 2 x 10^4 4 x 10^4
BM-MSCs	1 - 4 x 10^4	1 x 10^5 2 x 10^5 3 x 10^5 4 x 10^5

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 Freshly Isolated Human MSCs

The following protocol is for expansion of human MSCs from BM-MNCs or AD-SVF cells (prepared in section A or B) in a T-25 cm^2 flask. If using other cultureware, adjust cell numbers and volumes accordingly.

NOTE: Only use tissue culture-treated cultureware.

1. Seed freshly isolated MNCs or AD-SVF cells 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 MNCs/cm^2 (i.e. 1 - 2.5 x 10^6 cells per T-25 cm^2 flask)
   - For AD-derived cells: 6 - 10 x 10^3 AD-SVF cells/cm^2 (i.e. 1.5 - 2.5 x 10^5 cells per T-25 cm^2 flask)
2. Incubate at 37°C for 9 - 15 days until cells are approximately 80% confluent.
3. 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. Warm ACF Enzymatic Dissociation Solution and ACF Enzyme Inhibition Solution to room temperature (15 - 25°C). Do not incubate at 37°C.
   ii. Wash cells once with 2.5 mL of D-PBS.
   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 15 mL conical polypropylene tube.
   v. Wash the flask with 5 mL of complete MesenCult™-ACF Plus Medium and place into the tube from step iv.
   vi. Centrifuge the tube at 300 x g for 8 minutes with the brake on.
   vii. Discard the supernatant and resuspend the cell pellet in complete MesenCult™-ACF Plus Medium (see Notes and Tips).
   viii. Count viable cells using Trypan Blue and a hemocytometer.

Go to section E to further passage and expand cultured MSCs.

E) Expansion of Cultured Human MSCs

The following protocol is for expanding cultured MSCs (resulting cells at the end of section D, ES- or iPS-MPCs, and cryopreserved cultured MSCs from BM, AD or UC) 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 MSCs at a density of 1.5 - 4 x 10^3 cells/cm^2 (i.e. 4 - 10 x 10^4 cells/flask). For UC-MSCs, plate 1 - 6 x 10^3 cells/cm^2.
3. Incubate at 37°C until cells are approximately 80% confluent. This takes approximately 3 - 5 days.
4. Passage cells using Animal Component-Free Cell Dissociation Kit, following the instructions in section D, step 4.
5. Repeat step 4 as needed by plating MSCs at seeding densities recommended in section E, step 2.

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 (vii)].