MesenCult™-ACF Plus Umbilical Cord Culture Kit

Animal component-free medium and substrate for derivation and expansion of human mesenchymal stromal cells from umbilical cord tissue

Catalog #100-0234 1 Kit

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Product Description

MesenCult™-ACF Plus Umbilical Cord Culture Kit includes standardized, animal component-free (ACF) and serum-free media for the derivation and expansion of human mesenchymal stromal cells from umbilical cord tissue, also known as mesenchymal stem cells (UC-MSCs). MesenCult™-ACF Plus Umbilical Cord Medium supports the isolation of UC-MSCs and works together with complete MesenCult™-ACF Plus Medium for the long-term growth of UC-MSCs.

MesenCult™-ACF Plus Umbilical Cord (UC) 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 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 Umbilical Cord Culture Kit and 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 Medium. For a complete list of related products, including differentiation media available, visit www.stemcell.com, or contact us at techsupport@stemcell.com.

NOTE: L-Glutamine (Catalog #07100) is required for preparation of MesenCult™-ACF Plus UC Medium and complete MesenCult™-ACF Plus Medium; see Preparation of Reagents and Materials.

Product Information

The following components are available as part of a kit (Catalog #100-0234) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	QUANTITY	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™ Umbilical Cord Derivation 500X Supplement	100-0235	0.5 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
MesenCult™-ACF Plus 500X Supplement	100-0236	0.5 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
Animal Component-Free Cell Attachment Substrate	07130	3 x 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 #
L-Glutamine	07100
Gemtamicin, 50 mg/mL (recommended)	e.g. Sigma G1397
Human ab serum (optional)	e.g. Sigma H4522
0.5 mL screw cap polypropylene tubes	e.g. Sarstedt 72.785.005
Polyethylene Storage Bottle	38085
D-PBS (Without Ca++ and Mg++)	37350
Polypropylene conical tubes, 15 mL and 50 mL	e.g. 38009 and 38010
Tissue culture-treated 6-well plate	e.g. 38015
Animal Component-Free Cell Dissociation Kit • ACF Enzymatic Dissociation Solution • ACF Encyme Inhibition Solution	05426
Reversible strainers, 70 µm or 100 µm	e.g. 27260 or 27270
Trypan Blue	07050

Preparation of Reagents and Materials

MesenCult™-ACF Plus Umbilical Cord (UC) Medium

Use sterile technique to prepare MesenCult™-ACF Plus UC Medium (MesenCult™-ACF Plus Medium + MesenCult™ Umbilical Cord Derivation 500X Supplement + L-Glutamine + 50 mg/mL gentamicin [recommended] + human ab serum [optional]). The following example is for preparing 100 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw MesenCult™ Umbilical Cord Derivation 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. Do not exceed the shelf life of the supplement. After thawing the aliquots, use immediately. Do not re-freeze.
2. Combine components in a sterile polyethylene bottle as indicated in Table 1. Mix thoroughly.

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

Table 1. Preparation of MesenCult™-ACF Plus UC Medium

COMPONENT NAME	VOLUME
MesenCult™-ACF Plus Medium	96.2 mL
MesenCult™ Umbilical Cord Derivation 500X Supplement	200 µL
L-Glutamine	1 mL
50 mg/mL Gentamicin (recommended)	100 µL
Human ab serum (optional)	2.5 mL

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 100 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. Do not exceed the shelf life of the supplement. After thawing the aliquots, use immediately. Do not re-freeze.
2. Add 200 µL of MesenCult™-ACF Plus 500X Supplement to 98.8 mL of MesenCult™-ACF Plus Medium. Mix thoroughly.
3. Add 1 mL of L-Glutamine. Mix thoroughly.

NOTE: If not used immediately, store complete 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 (ACF) Cell Attachment Substrate.

NOTE: Use only tissue culture-treated cultureware.

1. Dilute ACF Cell Attachment Substrate in D-PBS (Without Ca++ and Mg++) as follows:
   - For isolating MSCs from umbilical cord tissue, dilute Attachment Substrate 1 in 50 in D-PBS.
     For example, add 120 µL of substrate to 5.88 mL of D-PBS.
   - For expansion of previously cultured MSCs from umbilical cord, dilute Attachment Substrate 1 in 150 in D-PBS.
     For example, to prepare a 1 in 150 dilution add 40 µL of substrate to 5.96 mL of D-PBS.

   Gently mix the diluted substrate solution. Do not vortex.
2. Immediately use the diluted substrate solution to coat cultureware. Refer to Table 2 for recommended coating volumes.

Table 2. Recommended Volumes for Coating Cultureware with Diluted ACF Cell Attachment Substrate

CULTUREWARE	VOLUME OF DILUTED ACF CELL ATTACHMENT SUBSTRATE
6-well plate	1 mL/well
T-25 cm^2 flask	2.5 mL/flask
T-75 cm^2 flask	6 mL/flask

3. Gently tilt the cultureware to spread the substrate solution evenly across the surface.
4. 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 for 30 minutes before proceeding to the next step.
5. 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.
6. Wash cultureware once using D-PBS (e.g. use 2 mL/well if using a 6-well plate).
7. Aspirate wash solution when tissue pieces or MSCs are ready to be plated.

Directions for Use

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

A. Isolation of Human UC-MSCs Using the Explant Method

B. Expansion of Human UC-MSCs

NOTE: The use of polypropylene tubes during subculture will help to prevent the MSCs from sticking to the tubes.

A. Isolation of Human UC-MSCs Using the Explant Method

The following protocol is for isolating MSCs from fresh (< 36 hours after birth) human umbilical cord using the explant method.

1. Coat T-75 cm^2 flasks with ACF Cell Attachment Substrate diluted 1 in 50 in D-PBS (see Preparation section).
2. Cut whole umbilical cord into ~5 cm pieces.
3. Carefully dissect out the vessels with surrounding Wharton's Jelly (WJ); try to avoid cutting into the vessel (to reduce contaminating red blood cells and endothelial cells).
4. Maintain moisture of the tissue by adding D-PBS dropwise to tissue periodically while dissecting (every 5 - 10 minutes).

   NOTE: For higher yield of MSCs it is recommended to only use the perivascular WJ, which contains the highest concentration of MSCs.
5. Carefully strip the perivascular WJ from the dissected vessels; perivascular WJ should peel away from the vessel in long strips. Transfer to a 50 mL conical tube containing a small amount of D-PBS (just enough to cover the tissue).
6. Discard the amnion and outer WJ (optional) and the stripped vessels.
7. Mince WJ tissue into ~1 - 3 mm^2 pieces using a scalpel, tissue chopper, or scissors, adding D-PBS periodically.
8. Add 1 g of minced tissue to each coated T-75 cm^2 flask. Cover with 8 mL of MesenCult™-ACF Plus UC Medium.
9. Incubate at 37°C undisturbed for 5 - 6 days before checking for growth. This allows the explants to attach to the surface.

10. Perform a half-medium change on day 6 or day 7 as follows:
    a. Carefully remove 4 mL of medium; do not disturb the explants that are attached. If there are some floating it is OK to remove them, but best to avoid them.
    b. Slowly add 5 mL of warm (37°C) MesenCult™-ACF Plus UC Medium.
    c. Incubate at 37°C.
11. Day 10 - 14: Many of the explants should have balled up and detached. This is an indicator that the cells that have migrated out from the explants are ready for harvest. If the colonies formed are beginning to detach or peel, this is also an indication that the cells are ready for harvest. Proceed to step 12.
12. Coat cultureware with ACF Cell Attachment Substrate diluted 1 in 150 in D-PBS (see Preparation section).
13. Remove medium and loose fragments from the flask. Discard.
14. Rinse flask with 4 - 5 mL D-PBS. Remove and discard D-PBS.
15. Detach cells using Animal Component-Free Cell Dissociation Kit, as follows:
    a. Thaw ACF Enzymatic Dissociation Solution and ACF Enzyme Inhibition Solution at 2 - 8°C. Mix thoroughly.

       NOTE: Once thawed, use immediately or store at 2 - 8°C for up to 4 days. Alternatively, aliquot and store solutions at -20°C. Do not exceed the shelf life of the solutions. After thawing the aliquoted solutions, use immediately. Do not re-freeze.
    b. Warm the ACF Enzymatic Dissociation Solution and ACF Enzyme Inhibition Solution to room temperature (15 - 25°C). Do not incubate at 37°C.
    c. Add 5 mL ACF Enzymatic Dissociation Solution to the flask. Incubate at 37°C for 6 - 7 minutes.
    d. Tap the bottom and sides of the flask to detach cells. If 10 - 20% of cells have not detached, incubate an additional 2 - 3 minutes.

       NOTE: Do not pipette up and down to release cells. If cells are not detached after 9 minutes of dissociation, add 5 mL of ACF Enzyme Inhibition Solution. Transfer detached cells to a 50 mL conical tube. Add 5 mL of complete MesenCult™-ACF Plus Medium to the remaining cells, then use a cell scraper to carefully release the remaining cells. Proceed to step f.
    e. Add 5 mL ACF Enzyme Inhibition Solution.
    f. Transfer detached cells to a 50 mL conical tube; do not pipette up and down.
    g. Rinse flask with 8 - 10 mL complete MesenCult™-ACF Plus Medium. Add the rinse to the conical tube.
16. Filter cells through a 70 µm or 100 µm reversible strainer into a new 50 mL conical tube to remove any remaining tissue pieces.
17. Centrifuge cells at 300 x *g* for 10 minutes.
18. Aspirate supernatant, then flick the tube to resuspend pellet; do not pipette up and down.
19. Perform a viable cell count using Trypan Blue and a hemocytometer.
20. Plate cells for expansion onto coated cultureware (prepared in step 12) as indicated in Table 3.

    NOTE: Do not freeze cells at the end of P0, as they are fragile and this will result in poor recovery post-thaw. If needed, freeze cells after P1.

Table 3. Recommended Plating Densities and Medium Volumes for Various Cultureware

COATED CULTUREWARE	EXAMPLE OF PLATING DENSITY*	MEDIUM VOLUME (mL)
6-well plate	1.0 - 6 x 10^4 cells/well	2
T-25 cm^2 flask	0.25 - 1.5 x 10^5 cells	5
T-75 cm^2 flask	0.75 - 4.5 x 10^5 cells	12

* It is recommended to plate two seeding densities within the range provided (e.g. 1.5 x 10^4 and 3 x 10^4 cells/well of a 6-well plate)

B. Expansion of Human UC-MSCs

The following protocol is for a single T-75 cm^2 flask. If using other cultureware, adjust cell numbers and volumes accordingly.

NOTE: Only use tissue culture-treated cultureware.

1. Coat a T-75 cm^2 flask with ACF Cell Attachment Substrate diluted 1 in 150 in D-PBS (see Preparation section).
2. Plate UC-MSCs in 12 mL of complete **MesenCult™-ACF Plus Medium**. Refer to Table 3 for recommended cell plating densities for various cultureware.
3. Incubate at 37°C until cells are ~80% confluent (see Figure 1). This takes approximately 3 - 5 days.

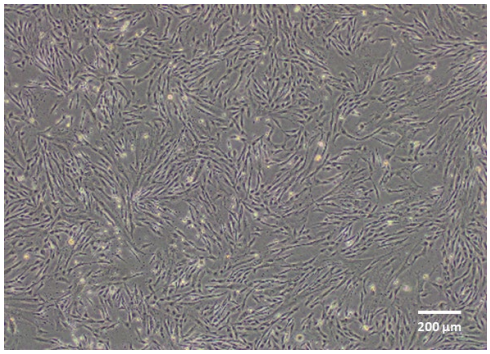

Figure 1. 80% Confluent UC-Derived MSCs (Passage 2) Derived and Expanded Using MesenCult™-ACF Plus Umbilical Cord Culture Kit

4. Coat cultureware with ACF Cell Attachment Substrate diluted 1 in 150 in D-PBS (see Preparation section).
5. Passage cells as follows:
   a. Wash cells with 5 mL D-PBS; remove and discard D-PBS.
   b. Add 5 mL ACF Enzymatic Dissociation Solution and incubate at 37°C for 5 - 7 minutes.
      NOTE: For the first passage following explant derivation, dissociation may require 5 - 9 minutes.
   c. Tap flask to release the cells.
   d. Add 5 mL ACF Inhibition Solution. Transfer cells to a 50 mL conical tube.
      NOTE: For the first passage following explant derivation, use a cell strainer to remove the tissue pieces.
   e. Wash flask with 10 mL complete MesenCult™-ACF Plus Medium to collect any remaining loosely attached cells and add to the 50 mL conical tube.
   f. Centrifuge at 300 x *g* for 7 minutes.
   g. Discard the supernatant, then resuspend cell pellet in complete MesenCult™-ACF Plus Medium.
6. Perform a viable cell count using Trypan Blue and a hemocytometer. Plate cells onto coated cultureware (prepared in step 4) as indicated in Table 3.
7. Repeat steps 1 - 6 as needed.