EC-Cult™-XF Culture Kit

Culture kit for derivation and proliferation of human endothelial colony-forming cells (ECFCs) and mature endothelial cells

Catalog #08000 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

EC-Cult™-XF Culture Kit is a xeno-free medium for the culture of mature human endothelial cells and endothelial colony-forming cells (ECFCs). It has been developed for the expansion of vascular (large, small, and lymphatic vessel-derived endothelial cells) and perivascular cells (human placental pericytes), as well as for the derivation and proliferation of ECFCs from human umbilical cord blood and peripheral blood. For complete instructions for the ECFC assay, refer to the Technical Manual: Culture of Human Endothelial Colony-Forming Cells (ECFCs) Using EC-Cult™-XF Culture Kit (Document #DX21400), available at www.stemcell.com or contact us to request a copy.

EC-Cult™-XF Medium must be used in conjunction with Animal Component-Free Cell Attachment Substrate (Component #07130; included in EC-Cult™-XF Culture Kit). For passaging, Animal Component-Free Cell Dissociation Kit (Catalog #05426) is required. EC-Cult™-XF Medium must be supplemented with Heparin Solution (Catalog #07980).

Product Information

The following components are sold as a complete kit (Catalog #08000) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
EC-Cult™-XF Basal Medium	08003	150 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
EC-Cult™-XF 2.5X Supplement*	08004	100 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.

*This component contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
Heparin Solution	07980
Polyethersulfone (PES) filter unit (0.2 - 0.22 µm)	e.g. Fisher 09-741-04 (0.2 µm, 250 mL) OR Fisher SCGP00525 (0.22 µm, 50 mL)
D-PBS (Without Ca++ and Mg++)	37350
Animal Component-Free Cell Dissociation Kit • ACF Enzymatic Dissociation Solution • ACF Enzyme Inhibition Solution	05426
Falcon® Conical Tubes, 15 mL	38009
Trypan Blue	07050

Preparation of Reagents and Materials

Complete EC-Cult™-XF Medium

Use sterile techniques to prepare complete EC-Cult™-XF Medium (Basal Medium + 2.5X Supplement + heparin). The following example is for preparing 250 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw the 2.5X Supplement either at 2 - 8°C overnight or at 37°C until fully thawed. Mix thoroughly but do not vortex.

   NOTE: Some precipitate may form. This will not affect product performance and will be removed when the complete medium is filtered (step 4).

   NOTE: Once thawed, use immediately or aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing the aliquots, use immediately. Do not re-freeze.
2. Warm EC-Cult™-XF Basal Medium and Heparin Solution to room temperature (15 - 25°C).
3. Prepare 250 mL of medium by combining components as indicated in Table 1.

Table 1. Preparation of Complete EC-Cult™-XF Medium for Various Cell Types

ENDOTHELIAL CELL TYPE	COMPONENT VOLUME (mL)		
	EC-Cult™-XF Basal Medium	EC-Cult™-XF 2.5X Supplement	Heparin Solution
ECFCs or pericytes	143.75	100	6.2 (final concentration 50 µg/mL)
HUVECs	148.45	100	1.55 (final concentration 12.5 µg/mL)
HAECs, HPAECs, dMVECs, or HDLECs	147	100	3.1 (final concentration 25 µg/mL)

ECFC: Endothelial colony-forming cell; HUVEC: Human vein endothelial cell; HAEC: Human aortic endothelial cell; HPAEC: Human pulmonary artery endothelial cell; dMVEC: Dermal microvascular endothelial cell; HDLEC: Human dermal lymphatic endothelial cell

4. Mix thoroughly. Filter the complete medium through a 0.2 - 0.22 µm PES filter unit.

   NOTE: If not used immediately, store complete EC-Cult™-XF Medium at 2 - 8°C for up to 15 days. If a precipitate forms, filter again as described above. This will not affect performance of the medium.

Coating Cultureware with Animal Component-Free (ACF) Cell Attachment Substrate (for mature endothelial cells and pericytes)

Use sterile techniques when coating cultureware with ACF Cell Attachment Substrate.

For instructions for coating cultureware for ECFCs, refer to the Technical Manual: Culture of Human Endothelial Colony-Forming Cells (ECFCs) Using EC-Cult™-XF Culture Kit (Document #DX21400), available at www.stemcell.com or contact us to request a copy.

NOTE: Only use tissue culture-treated cultureware.

1. Dilute ACF Cell Attachment Substrate in D-PBS (Without Ca++ and Mg++) (PBS) as indicated below:
   - For HUVECs: 1 in 400
   - For HAECs, HPAECs, dMVECs, HDLECs, or pericytes: 1 in 100
2. Gently mix diluted ACF Cell Attachment Substrate. Do not vortex.
3. Immediately use diluted ACF Cell Attachment Substrate 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
24-well plate	0.4 mL/well
6-well plate	1.0 mL/well
T-25 cm^2 flask	4 - 5 mL/flask
T-75 cm^2 flask	8 - 9 mL/flask

4. Gently rock cultureware back and forth to spread ACF Cell Attachment Substrate evenly across the surface. Seal with Parafilm®.

5. Incubate sealed cultureware at room temperature (15 - 25°C) for at least 2 hours before use. Do not let ACF Cell Attachment Substrate evaporate.

   NOTE: Sealed coated cultureware can be stored at 2 - 8°C for up to 3 days after coating. Allow stored coated cultureware to warm to room temperature (15 - 25°C) for 30 minutes before proceeding to step 6.
6. Gently tilt cultureware onto one side and allow excess ACF Cell Attachment Substrate to collect at the edge. Remove excess solution using a serological pipette or by aspiration. Ensure that the coated surface is not scratched.
7. Wash cultureware twice with PBS (e.g. use 2 x 2 mL/well if using a 6-well plate).
8. Aspirate PBS. The coated cultureware is now ready for use.

Directions for Use

For complete instructions for the ECFC assay, refer to the Technical Manual: Culture of Human Endothelial Colony-Forming Cells (ECFCs) Using EC-Cult™-XF Culture Kit (Document #DX21400), available at www.stemcell.com or contact us to request a copy.

The following protocols are for passaging, expansion, cryopreservation, and thawing of mature endothelial cells (ECs) and pericytes.

A. PASSAGING AND EXPANSION

Passage ECs and pericytes at 90% confluency; refer to Figure 1 for a representative image.

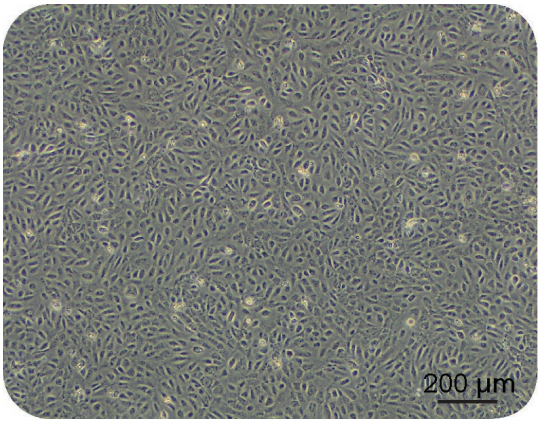

Figure 1. Monolayer of HUVECs at Passage 2

1. Coat the desired culture vessel(s) for expansion (e.g. T-25 cm^2 or T-75 cm^2 flask) with ACF Cell Attachment Substrate (see Preparation section).
2. Detach cells using the Animal Component-Free Cell Dissociation Kit as follows:
   a. Warm ACF Enzymatic Dissociation Solution and ACF Enzyme Inhibition Solution to room temperature (15 - 25°C). Do not incubate at 37°C.
   b. Wash the well or flask twice with PBS.
   c. Add ACF Enzymatic Dissociation Solution to the well or flask according to Table 3.

Table 3. Recommended Volume of ACF Enzymatic Dissociation Solution or ACF Enzyme Inhibition Solution for Various Cultureware

CULTUREWARE	VOLUME OF ACF ENZYMATIC DISSOCIATION SOLUTION OR ACF ENZYME INHIBITION SOLUTION
24-well plate	0.2 mL/well
6-well plate	0.5 mL/well
T-25 cm^2 flask	1 mL/flask
T-75 cm^2 flask	2 mL/flask

   d. Incubate at 37°C for 4 - 5 minutes.
   e. Gently tap the cultureware to detach cells. If < 90% of cells have detached, incubate at 37°C for an additional 1 - 2 minutes and tap again.

f. Add ACF Enzyme Inhibition Solution to the well or flask according to Table 3.

g. Pipette up and down to create a single-cell suspension and to lift any remaining cells. Add the cell suspension from one well or flask to one 15 mL conical tube.

h. Wash the well or flask with PBS and add the wash to the corresponding tube from step g.

i. Centrifuge the cell suspension at 300 x *g* for 5 minutes with the brake on.

j. Remove and discard the supernatants. Resuspend each cell pellet in complete EC-Cult™-XF Medium as follows:

- For one well of a 6-well plate, resuspend in 0.5 mL
- For a T-25 cm^2 flask, resuspend in 1 mL
- For a T-75 cm^2 flask, resuspend in 2 mL

3. Perform a viable cell count using Trypan Blue and a hemocytometer.
4. Add cells to coated plates or flasks at the desired density in complete EC-Cult™-XF Medium. The optimal density is 10,000 cells/cm^2 for cells to reach confluency in 2 - 4 days.

   NOTE: Appropriate seeding densities range from 2,500 - 15,000 cells/cm^2 and may require optimization depending on the proliferative potential of each sample. At a seeding density of 10,000 cells/cm^2, HUVECs reach confluency in 2 - 5 days, averaging 3 days between passages.
5. Incubate at 37°C and 5% CO_2. If the cells have not grown to confluency after 3 days, perform a full medium change.

B. CRYOPRESERVING CELLS

The following protocol is for cryopreserving ECs or pericytes using Cryostor® CS10 (Catalog #07930), a serum-free, animal component-free, defined cryopreservation medium.

1. Detach cells as described in section A step 2.
2. Perform a viable cell count using Trypan Blue and a hemocytometer.
3. Resuspend cells to a concentration of 0.5 - 1 x 10^6 cells/mL in cold (2 - 8°C) CryoStor® CS10. Mix thoroughly and transfer the suspension to a cryovial.
4. Cryopreserve cells using a standard slow rate-controlled cooling protocol (approximately -1°C/minute) or an isopropanol freezing container.
5. After 24 hours, transfer cryovials to liquid nitrogen for long-term storage (-135°C).

   NOTE: Long-term storage at -80°C is not recommended.

C. THAWING CELLS

The following protocol is for thawing one vial of cryopreserved ECs or pericytes into a tissue culture flask. This protocol may be used for cryopreserved cells previously expanded in either serum-containing medium or EC-Cult™-XF Medium.

1. Coat a T-75 cm^2 tissue culture flask with ACF Cell Attachment Substrate (see Preparation section).
2. Warm a 1 mL aliquot and a 2 mL aliquot of complete EC-Cult™-XF Medium in 15 mL conical tubes in a 37°C water bath or incubator. Place tubes in a biosafety cabinet along with the coated flask.

   NOTE: Ensure this step is completed before proceeding to step 3.
3. Remove a vial of 1 x 10^6 cryopreserved cells from liquid nitrogen storage and immerse up to 2/3 of vial height in a 37°C water bath. Gently swirl until there is only a small amount of ice remaining (approximately 30 - 60 seconds). Avoid shaking the vial.
4. Wipe the outside of the vial with 70% ethanol or isopropanol prior to placing it in the biosafety cabinet.
5. Using a 1 mL pipette, slowly (dropwise) transfer thawed cell suspension to the 15 mL conical tube containing 1 mL of warm medium (prepared in step 2). Rinse the vial with the additional 2 mL of warm medium and add the wash to the 15 mL tube. Mix by gently swirling.

   NOTE: Do not centrifuge the cells.
6. OPTIONAL: Perform a viable cell count using Trypan Blue and a hemocytometer.
7. Add cells to the coated flask (prepared in step 1) at a density of > 10,000 cells/cm^2 (e.g. 1 x 10^6 cells per T-75 cm^2 flask). Do not exceed a density of 15,000 cells/cm^2.
8. Incubate at 37°C and 5% CO_2.
9. After 18 - 24 hours, remove medium and replace with fresh complete EC-Cult™-XF Medium.

Related Products

For related products, including specialized culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com or contact us at techsupport@stemcell.com.