EC-Cult™-XF ECFC Culture Kit

Culture kit for derivation and proliferation of human endothelial colony-forming cells

Catalog #08000 1 Kit

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Product Description

EC-Cult™-XF ECFC Medium is a xeno-free medium for the culture of human endothelial colony-forming cells (ECFCs). It has been specifically developed for the derivation and proliferation of ECFCs from human umbilical cord blood and human adult peripheral blood. It is optimized for isolation and counting of ECFCs using a 7- to 14-day ECFC assay. EC-Cult™-XF ECFC Medium supports long-term expansion of ECFCs, and cells maintain their clonogenic properties along the entire ECFC hierarchy.

EC-Cult™-XF ECFC Medium must be used in conjunction with Animal Component-Free Cell Attachment Substrate (Component #07130; included in EC-Cult™-XF ECFC Culture Kit). For passaging ECFCs, Animal Component-Free Cell Dissociation Kit (Catalog #05426) is required. EC-Cult™-XF ECFC 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 ECFC Basal Medium	08001	300 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
EC-Cult™-XF ECFC 2.5X Supplement*	08002	200 mL	Store at -20°C.	Stable for 24 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.

*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.

Preparation of Complete EC-Cult™-XF ECFC Medium

Use sterile techniques to prepare complete EC-Cult™-XF ECFC Medium (Basal Medium + 2.5X Supplement + heparin). The following example is for preparing 500 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw the 2.5X Supplement 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 5).

   NOTE: Once thawed, use immediately or aliquot and store at -20°C for up to 10 months. Do not exceed the shelf life of the supplement. After thawing the aliquots, use immediately. Do not re-freeze.
2. Warm EC-Cult™-XF ECFC Basal Medium and Heparin Solution (Catalog #07980) to room temperature (15 - 25°C).

   NOTE: If using a new bottle of Basal Medium, remove 12.5 mL from the original 300 mL volume.
3. Add 200 mL of 2.5X Supplement to 287.5 mL of Basal Medium.
4. Add 12.5 mL of Heparin Solution (final concentration 50 µg/mL). Mix thoroughly.
5. Filter the complete medium through a 0.2 - 0.22 µm polyethersulfone (PES) filter unit (e.g. Fisher 09-741-04 [0.2 µm, 250 mL]; Fisher SCGP00525 [0.22 µm, 50 mL]).

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

Coating Cultureware with Animal Component-Free (ACF) Cell Attachment Substrate

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

NOTE: Only use tissue culture-treated cultureware.

1. Dilute ACF Cell Attachment Substrate 1 in 100 in D-PBS (Without Ca++ and Mg++) (PBS).

   For example, add 100 µL of ACF Cell Attachment Substrate to 9.9 mL of PBS.

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 1 for recommended coating volumes.

Table 1. 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.
5. Incubate at room temperature (15 - 25°C) for at least 2 hours before use. Do not let ACF Cell Attachment Substrate evaporate.

   NOTE: If not used immediately, the cultureware must be sealed to prevent evaporation of ACF Cell Attachment Substrate (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 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 using 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.
   - For ECFC derivation, plates must be coated and used on the same day as cord blood processing.
   - For established ECFC cultures, store coated cultureware at 2 - 8°C for up to 3 days prior to 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 ECFC Medium (Document #DX21400), available at www.stemcell.com or contact us to request a copy.

Related Products

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