Product Information

Endothelial Cell Culture Reagents

■ **CS-C MEDIUM WITH SERUM FOR ENDOTHELIAL CELL LINES (Product No. C 1431)**
■ **CS-C MEDIUM WITHOUT SERUM FOR ENDOTHELIAL CELL LINES (Product No. C 1556)**
■ **ENDOTHELIAL CELL ATTACHMENT FACTOR (ECAF) (Product No. E 9765)**
■ **ENDOTHELIAL CELL GROWTH FACTOR (100X) (Product No. E 9640)**
■ **TRYPSIN/EDTA SOLUTION FOR ENDOTHELIAL CELL CULTURES (1X) (Product No. T 4299)**

DIRECTIONS FOR THAWING HUMAN ENDOTHELIAL CELLS

1) Frozen cells should be stored under liquid nitrogen.
2) Rapidly thaw the cells by immersion in a 37°C water bath.
3) Immerse in 70% ethanol. Dry with sterile gauze.
4) Transfer contents to a 15 ml centrifuge tube. Add 1.0 ml CS-C medium (C 1431/C 1556) and mix gently.
5) Wait one minute, add 2.0 ml CS-C medium and mix gently.
6) Wait one minute, add 4.0 ml CS-C medium and mix gently.
7) Pellet cells by gentle centrifugation. Aspirate supernatant, leaving a minimal volume to cover cells. Loosen pellet by flicking sharply with fingers.
8) Add Endothelial Cell Growth Factor (E 9640) to loosened cell pellet at 1% of the final culture medium volume. Mix gently.
9) Add CS-C medium to final volume. Mix gently.
10) Plate cells on Endothelial Cell Attachment Factor (E 9765) coated tissue culture surface.

PASSAGE OF ENDOTHELIAL CELL CULTURES

1) Remove medium from endothelial cells and replace with sufficient Trypsin-EDTA solution (T 4299) to cover the cell layer. Washing the cell layer with PBS or EDTA before adding the protease is optional.
2) Incubate at 37°C until the cells are well rounded and begin to detach. Some cells may detach more quickly than others as a function of where they are in the cell cycle and the amount of extracellular matrix present.
3) Cells may be harvested after rounding by sharply rapping the culture flask with the hand or against a hard surface.
4) Add an equal volume (or a minimum of 5 ml) of Trypsin Inhibitor solution (T 0800) to the cell suspension and transfer to a sterile centrifuge tube.
5) Rinse culture flask with CS-C medium (C 1431/ C 1556) to remove remaining cells. Add cells to the centrifuge tube. Pellet cells by gentle centrifugation. Aspirate supernatant, leaving a minimal volume to cover cells.
6) Loosen the cell pellet by flicking the centrifuge tube with fingers. Do not vortex.
7) Add Endothelial Cell Growth Factor (E 9640) to the loosened pellet at 1% of the final culture medium volume. Mix gently.
8) Adjust cell concentration for plating or counting.
9) Inoculate cells on an Endothelial Cell Attachment Factor
(E 9765) coated tissue culture surface. Incubate at 37°C, 5% CO_2, and 100% humidity.
10) Feed cultures CS-C medium every two days when actively proliferating.
11) Add Endothelial Cell Growth Factor directly to refed cultures (1% v/v) or to CS-C medium (1% v/v) just prior to feeding cells. Adding the growth factor to an entire bottle of CS-C medium is **not recommended.**

■ ENDOTHELIAL CELL ATTACHMENT FACTOR (ECAF) (Product No. E 9765)

ECAF is optimized to promote the attachment, spreading and polarity of endothelial cells. It contains gelatin (in HEPES buffered medium) to which factors separated from serum by chromatography and dialysis have been added.

PRODUCT USE

1) ECAF should be thawed and warmed to 37°C before use. Cover the tissue culture surface to be plated for 1 minute.
2) Aspirate coating solution. Cells can be plated immediately.
3) ECAF can (if desired) be moved from one well of a multiwell plate to another, or from one flask to another without reducing its effectiveness.
4) Store ECAF at –70°C. Shelf life at 2-8°C is two weeks