



**1. What growth medium is used to culture endothelial and smooth muscle cells?**

Please refer to the “Culture Protocol” tab of the individual sample’s homepage on the Catalog (<https://www.coriell.org/>) for the details on what culture medium is required for the sample you have purchased. If supplements are indicated please see below:

Heparin, Porcine intestinal mucosal, Na salt (Sigma-Aldrich #H-3149)

Endothelial Cell Growth Supplement (ECGS):

1) Partially purified: 20 µg/ml (Sigma-Aldrich #E0760 or E2759)

2) Purified ECGS or acidic FGF: 10-20ng/ml (Peprotech, Inc. #100-17A; Sigma No. F5542)

**2. Should a culturing substrate be used?**

Please refer to the “Culture Protocol” tab of the individual sample’s detail page on the Catalog (<https://www.coriell.org/>) to determine if a substrate should be used. If Gelatin or Fibronectin are indicated, please see below.

Gelatin, use 1% Gelatin (Sigma-Aldrich, #G1890) in 0.9% sodium chloride.

Fibronectin, use 1.5 µg/cm<sup>2</sup> human fibronectin (Millipore Corp. #FC010-10MG)

**3. What trypsinization solutions are needed?**

-0.53mM EDTA in HBSS (Gibco Versene Solution #15040-066 or equivalent)

-0.04% Trypsin/0.53 mM EDTA (Gibco Trypsin #25300-054 or equivalent)

**4. How are endothelial and smooth muscle cell lines subcultured?**

Volumes are for 25 cm<sup>2</sup> flasks

1) Culture must be subcultured when confluent (usually 5-7 days).

2) Remove medium.

3) Rinse cell monolayer with 3 ml of EDTA solution.

4) Remove EDTA solution.

5) Add 1 ml of Trypsin–EDTA solution to flask and incubate for 2-7 minutes or until cells start to round up and come off surface.

6) Add 5 ml of growth medium to flask.

7) Gently triturate cells and medium to obtain single-cell suspension.

8) Remove 0.5 ml aliquot to count.

9) If necessary, aspirate substrate solution from new flask.

10) Inoculate flasks at  $(0.5-1) \times 10^4$  cells/cm<sup>2</sup>.

11) Gently move flask back and forth in perpendicular directions to evenly distribute cells.

12) Incubate flasks at 37°C in 5% CO<sub>2</sub> incubator.

**5. What is the freezing medium used to cryopreserve endothelial and smooth muscle cell lines?**

Growth medium + 15% FBS + 5% DMSO

**6. How should endothelial and smooth muscle cell lines be cryopreserved and stored?**



1. Place cells into a single-cell suspension, count and pellet as indicated in the subculture protocol above.
2. Resuspend the cells in freezing medium to a seeding density of  $5.0 \times 10^5$  viable cells per ml
3. Aliquot 1 ml into each cryovial or ampule.
4. Cells resuspended in freezing medium should be immediately placed in a controlled rate freeze machine that reduces temperature at a controlled rate of  $-1^\circ\text{C}/\text{min}$ . Alternatively, cryovials can be placed in an ethanol bath at  $-80^\circ\text{C}$  overnight before being placed in liquid nitrogen vapor.
5. Frozen cell stocks are stored in liquid nitrogen tanks. Glass ampules are submerged in liquid, plastic cryovials are stored in vapor phase.

\*Suppliers of reagents are listed for the convenience of culture recipients only. Such lists are not intended to be either selective or exhaustive, and Coriell Institute does not recommend specific products or suppliers. Other media and reagents may be satisfactory, but have not been tested.