

Endothelial Cell Culture Kit V-1, Porcine

(Porcine Cryopreserved Cell and culture medium)

Cat. No. PMC-ENC01-COS

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Principle

Endothelial cells of ENC01 are isolated from porcine aorta. They are characterized by acetylated LDL uptake. The kit contains cryopreserved endothelial cells and culture medium.

Components/Storage

Components	Size	Quantity	Storage Conditions	Shelf Life
Endothelial Cells, Porcine	5 x 10 ⁵ cells/vial	1	Liquid Nitrogen	6 months
Culture Medium (Code: ENCM)	250 ml	1	-20°C Freezer	6 months

※ Shipping: dry ice

Components of Media:

ENCM is a complete medium designed for optimal culture of porcine endothelial cells in vitro. They are sterile, liquid basal medium (DMEM) which contain essential and non-essential amino acids, vitamins, other organic compounds, trace minerals, inorganic salts, FBS, and antibiotics.

Materials required but not provided

- Variable volume pipettes
- 15 ml centrifuge tube
- 25 cm² flask

Precautions

- Read the instructions carefully before beginning the culture.
- This kit is for research use only, not for human or diagnostic use.
- Always wear gloves and lab coat when handling the cell culture.

Protocols

1. Thaw the Culture Medium (Code: ENCM) in a 37°C water bath with gentle shaking.
2. Add 10 mL of Culture Medium into a 15ml centrifuge tube.
3. Quickly thaw the endothelial cell vial in a 37°C water bath. (for 1min 15sec)
4. Transfer thawed cells into a 15 ml centrifuge tube containing 10 ml of Culture Medium and mix gently. Centrifuge for 5 minutes at 4°C at 200 g.
5. After removing the supernatant, resuspend cells in 10 ml of the medium.
6. Centrifuge for 5 minutes at 4°C at 200 g. After removing the supernatant, resuspend cells in 10 ml of the medium.
7. Dispense 5 ml of cell suspension to 25 cm² flasks.
If seeding into well plates or dishes, the recommended density is 1.0-1.5 x 10⁴ cells / cm².
8. Incubate the plate at 37°C under 5% CO₂, 100% humidity for overnight.
9. Change 5 ml of Culture Medium the day after seeding and every other day thereafter.
10. Endothelial cell culture becomes 80% within 2 - 3 days.

Subculture

1. Subculture the cells when they are 80% confluent.
2. Rinse twice the cells with room temperature Hank's BSS or PBS.
3. Replace 3 ml of trypsin/EDTA solution (0.05% trypsin, 0.53mM EDTA) into flask and remove it immediately.
4. Cells are covered by trypsin/EDTA solution;
continue trypsinization until cells are completely rounded up (monitored with inverted microscope).
Caution: If only a few cells detach, warm the flask at 37°C .

We do not recommend continuing over 15 minutes of trypsinization.

5. Add Culture Medium to stop of the trypsinization,
and then transfer the cells into a centrifuge tube.
Centrifuge for 5 minutes at 4°C at 200 g.
6. After removing the supernatant, add culture medium.
Count cell and plate cells in a new culture vessel.

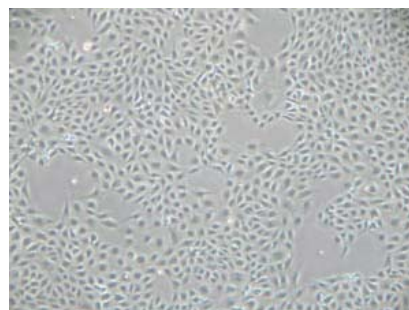


Fig. 1 Morphology of endothelial cells



COSMO BIO CO., LTD.

TOYO EKIMAE BLDG. 2-20, TOYO 2-CHOME,
KOTO-KU. TOKYO 135-0016, JAPAN
TEL : (81)3-5632-9617
FAX : (81)3-5632-9618
e-mail : export@cosmobio.co.jp
URL : www.cosmobio.com

