

Protocol for use No. 74: upcyte® HUVECs & Medium

This protocol describes how to prepare upcyte® Endothelial Cell Culture Medium and how to culture upcyte® Human Umbilical Vein Endothelial Cells (HUVECs).

Required products for upcyte® HUVEC culture

(A) Endothelial Cell Culture Medium

Our Endothelial Cell Culture Medium is suitable for culture of primary human endothelial cells and upcyte® HUVECs. It consists of the following components:

- 500 mL basal medium
- 25 mL fetal bovine serum (FBS)
- 29.2 mL L-glutamine
- 2.5 mL supplement A
- 1.5 mL supplement B

Store the basal medium protected from light at 2-8 °C. Do not freeze the basal medium. Store FBS, L-glutamine and the supplements A/B at -20 °C. If stored properly, single components are stable as indicated by the expiry date on the label.

For preparation of the complete medium, thaw FBS, L-glutamine and supplements A/B at room temperature. Transfer the entire content of each compound to the basal medium. Close the bottle, swirl gently and store at 2-8°C. After combining all components, the medium is stable for 6 weeks.

(B) upcyte® Human Umbilical Vein Endothelial Cells

Upon arrival, store upcyte® HUVECs in liquid or vapor phase nitrogen. They should not be stored at -70°C. After thawing, we recommend a pre-culture of 4-5 days to ensure sufficient recovery of the cells.

(C) Additional products not supplied:

- PBS without Ca²⁺ or Mg²⁺
- Trypsin-EDTA (0.05% Trypsin / 0.02% EDTA)

- cell culture vessels and disposables

Culture protocol

Step 1: thawing of cells

1. Remove the cells from the storage tank.
2. Thaw cells at 37°C in a water bath for approximately 120 sec. A small piece of ice should still be visible. Disinfect the vial using 70% ethanol and transfer the vial to a laminar flow-hood.
3. Transfer the thawed cell suspension into a tube containing 10 mL of pre-warmed medium.
4. Wash the cryovial once with 1 mL of medium to maximize cell recovery.
5. Pellet the cells by centrifuging at 250 x g for 5 min at RT.
6. Aspirate the supernatant without disrupting the pellet.
7. Add an appropriate volume of pre-warmed medium to the pellet (2 mL per 2E6 cells) and carefully resuspend the cells.
8. Determine the cell number e.g. by using a Neubauer haemocytometer.
9. Seed the cells at 1E4 cells per cm². Use ~0.2 mL medium per cm² surface area.
10. Incubate at 37 °C and 5% CO₂.
11. Change the medium every 2-3 days.
12. Split the cells when reaching 70-80% confluence, usually achieved within 4-5 days.

Step 2: plating of cells

1. Pre-warm culture medium to 37°C.
2. Aspirate the medium from the culture flask.
3. Wash the flask once with PBS.
4. Aspirate PBS and add an appropriate volume of 0.05% trypsin/EDTA solution per culture dish (~20 µL per cm²).
5. Incubate 3-5 min at 37°C until most of the cells are rounded up (check under the microscope).
6. Gently tap the cell culture vessel to detach remaining cells.

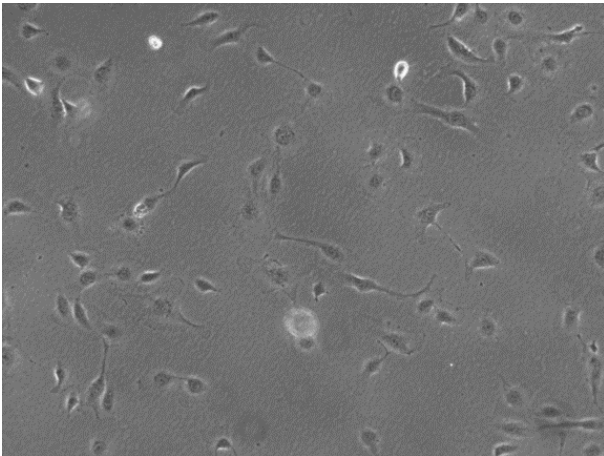
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7. Add an equal volume of pre-warmed medium and rinse the culture surface.
8. Transfer the complete suspension to a tube and centrifuge for 5 min 250 x *g* at RT.
9. Discard supernatant and carefully resuspend the pellet in medium.
10. Determine the cell number as described in the previous section.
11. Seed 20 000 cells per cm² in your format of choice.

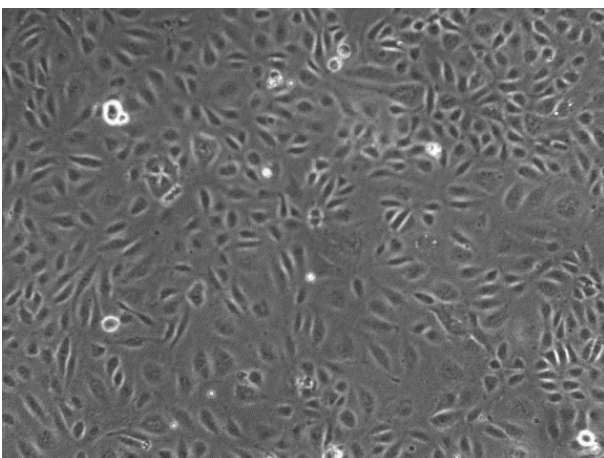
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Morphology of upcyte® HUVECs

Cells initially show marked spreading but become smaller and compact at higher cell densities.



Morphology of upcyte® HUVECs one day after thawing



Morphology of upcyte® HUVECs after 3 days of culture

Product information

Product	Supplements/Components	Product number
Endothelial Cell Culture Medium	<ul style="list-style-type: none">• Basal Medium (500mL)• FBS (25 mL)• L-Glutamine (29.2 mL)• Supplement A (2.5 mL)• Supplement B (1.5 mL)	MEC003
upcyte® Human Umbilical Vein Endothelial Cells (HUVECs)	<ul style="list-style-type: none">• 1 vial (2x10⁶ cells, cryopreserved)	CUV001

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