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Human Umbilical Vein Endothelial Cells

User protocol for the culture of human umbilical vein endothelial cells

- All cell culture procedures must be conducted in a bio-safety cabinet.
- Use aseptic technique to prevent microbial contamination.
- Cryo-preserved cells must be stored in liquid nitrogen or seeded immediately upon arrival.

⚠ Although medium is supplied sterile, please make sure to handle it under sterile conditions.

Prepare the Medium and culture flasks: (Please check which media is recommended on the concerning data sheet of your cells)

1. Warm the Basal Medium and Supplement(s) to 37°C
2. Please wipe the outside of all bottles with 70% alcohol to sterilize (see below)
3. Add Supplement(s) to the Basal Medium
4. Keep Growth Medium at room temperature.
5. Coat flasks with 2 ml Speed Coating Solution (SCS) per T25 flask and make sure whole surface is covered with SCS. Five minutes later dispose SCS by aspiration. Rinse the T25 flask with 5 ml PBS **(Room Temperature, RT**) twice. Flasks are now ready to be used.
6. Pre-equilibrate the culture flasks with 15 ml medium at 37°C, in a 5% CO_2 humidified incubator.

⚠ The supplemented medium can be stored at 4°C up to 4-6 weeks!
⚠ Never keep the medium for a longer period at 37°C. This might lead to glutamine degradation!

Thaw the Cells: **Please check the data sheets of cells for the correct seeding density, best seeding density for ECs after thawing is 20.000 cells/cm²!**

1. Place the vial of frozen cells in a 37°C water bath for 1 minute with gentle swirling of sample until only a little bit of ice is visible.
   Ensure that the vial is not completely submerged and water does not enter the vial.
2. Remove the vial from the water bath and wipe with 70% isopropanol or ethanol to sterilize.
3. Open vial and gently pipette the cell suspension up and down to evenly suspend cells.
4. Pipette the cells into 10 ml of full endothelial cell media in a 15 ml falcon tube and spin the cells down at 200 x g for 5 minutes.
5. Discard the supernatant and resuspend the cells gently with 10ml full endothelial media and transfer ALL cells into 1-2 T25 flask and culture the cells at 37° C in a CO_2 incubator.
6. Examine the cells microscopically to check even distribution of cells in each flask, and transfer to a CO_2 incubator. Do not disturb culture for the next 16 hours to allow cell attachment.
7. Medium replacement after 24 hours, replace medium over a cell free surface of the flask (please use only pre-warmed and pre-equilibrated medium) and add fresh medium 5ml per T25 or 15 ml per T75 over a cell free area of the flask. Never replace or add medium over the cell layer.
8. Re-feed cells every 48 hours until they become confluent. Increase medium volume to 7,5 ml if cells are between 20-45 % confluent and increase to 10 ml, if cells are more than 45 % confluent.

⚠ Normally cells become confluent within 7 days when seeded with 10.000 cells per cm^2.

⚠ Preparation of arrested cells:
Use Endothelial Basal Medium containing 0,5 % FBS. This will induce arrested endothelial cells after 18-24 hours.

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Passaging of primary cells:

For the detachment of the cells Trypsin / EDTA, TrypLE or Accutase can be used. Below please find a summarized overview of their properties.
Accutase: (Cat# PB-PAACCUTASE) is a mixture of proteolytic and collagenolytic enzymes isolated from crustacean; is mammary component free; for some cell types no need for inactivation or removal during passaging; is very gentle for cells, thus most of the surface proteins are intact after passaging; has to be stored at 4°C; Accutase is inactivated automatically after 1 h at 37°C.

Trypsin / EDTA: (PB-900028 – Passage Pack) is a solution of proteolytic enzymes containing Trypsin, Chymotrypsin and Elastase isolated from porcine pancreas that shows lot-to-lot variability in activity; is commonly used in cell culture; is available from different suppliers; has to be inactivated (e.g. FCS alone, FCS supplemented medium or trypsin inhibitor from soybean); compared with Accutase more cell culture experience of the user is necessary, because cells can be irreversibly damaged by too high trypsin concentrations or too long incubation times.

Passage of Cells (PB-PAACCUTASE):

1. Cells should be passaged when they reach 70 to 80% confluency (depending on the cell type). Do not allow the cells to become 100% confluent.
2. Aspirate the primary cell culture medium from the culture vessel.
3. Wash the cells by adding 100 µL / cm^2 of PBS (without calcium and magnesium), rotate the culture vessel carefully to rinse the cells, then aspirate the PBS.
4. Add 50 µL / cm^2 of detachment enzyme (Accutase is recommended) to the cells.
5. Incubate at room temperature until cells are rounded and start to detach. Check for cell detachment under the microscope!
6. Then tap the culture vessel.
7. When using Accutase: add two volumes of primary cell culture medium to the detached cells (or PBS w/ 2 % FBS).
8. Rinse the bottom of the culture vessel 2 to 3 times with the suspension to remove all cells and to separate them into a single cell suspension.
9. Transfer the cell suspension to a centrifugation tube.
10. Centrifuge the cells 5 min, 200 x g at room temperature.
11. Aspirate supernatant and resuspend the pellet in 1 to 2 mL primary cell culture medium.
12. Count the cells and seed viable cells according to the seeding density listed in the data sheet.

Passage of Cells (PB-900028 Passage Pack or PB-090K – Subculture Reagent Kit):

1. Remove and discard primary cell culture medium from the T-25 flasks.
2. Wasch cells twice with 10 ml HBSS.
3. Use warm (37°C) Trypsin-EDTA (1X) solution and add 2 ml to a T-25 flask.
4. As soon as cells have detached **(the flask may require a few firm gentle taps)**, add 5 ml Trypsin Neutralization Solution or add 10 ml of primary cell culture medium to the flask (medium supplemented with 5-10 % FBS will neutralize the trypsin).

⚠ **Over trypsinisation causes irreversible damage to cells**

5. Centrifuge 15 ml tube containing the cells at 200 x g for 5 minutes at room temperature.
6. After centrifugation, decant supernatant and discard.
7. Resuspend cell pellet in a known volume (~ 1 ml) of primary cell culture medium.
8. Count the cells using Tryphan Blue exclusion.
9. Count the cells and seed viable cells according to the seeding density listed in the data sheet
10. Add 5 ml of primary cell culture medium to a T25 flask. Increase medium volume to 7,5 ml if cells are between 20-45 % confluent and increase to 10 ml, if cells are more than 45 % confluent.
11. Change medium every two days.

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Cell Freezing procedure:

Materials:

- · Phosphate Buffered Saline (PBS)
- · Passage Pack (PELOBIOTECH Cat#: PB-900028 or PB-09K)
- · Tissue Culture Media
- · Cold Freezing Media (10% dimethylsulfoxide, DMSO and 10% FBS, and 80% culture basal medium), as alternative you can also use CryoStor Freezing media (PB-210102 – CryoStor10). Please ask for the CryoStor protocol.
- · Labeled Cryovials (~3 -5 per T-25 flask)
- · T25 flask or T75 flask confluent cells
- · CoolCell™ or CoolCell™ LX

⚠ **All media have to be chilled to 4°C!**

1. Flush the adherent layer with a 5 ml sterile pipette 3-5 times to dislodge loosely attached cells.
2. Remove and discard the cell culture media from the flask.
3. Wash adherent cells 2-3 times with 10 ml of sterile PBS (1X) without calcium and magnesium to remove nonadherent cells or fraction.
4. Remove and discard the wash solution from the flask.
5. Incubate cells with warm (37°C) Trypsin-EDTA (1X) solution (Use 2.0-3.0 ml of Trypsin-EDTA solution when collecting cells from T75 flasks, and 1.5-2.0 ml when using T25 flasks.) for 1-3 minutes or until cells are getting round and start to detach. Control detach process carefully to avoid irreversible damage of the cells. As soon as cells have detached (**the flask may require a few firm gentle taps**) (please control detachment under the microscope) add 10 ml cell culture medium supplemented with 5-10 % FBS, or 5 ml Trypsin Neutralizing solution. Prevent too long exposure to trypsin.
   Using Accutase please follow the concerning protocol.
6. Centrifuge the cell suspension at 200 g for 5 minutes.
7. Remove supernatant with sterile Pasteur pipette and resuspend cells in 1 ml cold cryopreservation media at 500,000 to 2,000,000 cells per ml into 4°C pre-cooled cryo tubes.

⚠ **Please ask for our detailed cryopreservation protocol (A: self-made cryo-medium or B: CryoStor 10)!**

8. Place the cells on ice, then take a sample to perform a cell count.
9. Place vials in BioCision "CoolCell" or other adequate cooling tools and place CoolCell freezing container at -70-80 °C for 24 h.
10. Transfer cryo tubes into your liquid nitrogen storage container.

⚠ **There is no need to pre-cool BioCision "CoolCell" and you do not need any alcohol!**

CoolCell™ and CoolCell™ LX Quick start

The 12 chambers and sample tubes should be dry to avoid tube sticking upon freezing.

⚠ **Make sure the core (black ring) is at room temperature and seated in the bottom of the central cavity.**

1. Place sample vials containing l ml of cell Suspension in each well. The sample vials should not extend above the CoolCell body. Check that the tubes slide in and out freely.
2. Fully seat the lid on the CoolCell.
3. Put the CoolCell upright into a -80°C freezer or dry ice locker. Ensure that there is at least l inch of free space clearance around the CoolCell.
1. Freeze for 4 hours before transferring samples to archive storage.

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Transferring frozen samples to archive storage

1. Prepare an insulated pan or Container with a l inch (2.5 cm) layer of pulverized or pellet dry ice.
2. Remove the CoolCell from the freezer and gently remove the lid using a twisting and rocking motion.
3. Immediately invert the CoolCell over the dry ice to recover the frozen vials. Check the CoolCell vial chambers to ensure that all chambers are clear. If any vials have stuck, release the vials by tapping the inverted CoolCell on a flat surface or on the palm of your hand.

SPECIAL NOTES:

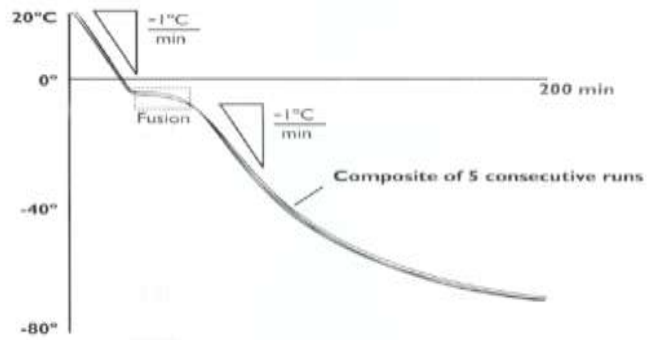
Always use dry ice to transfer the cells to permanent storage to avoid temperature rise and cell damage. Cryovial contents can rise from -80°C to over -50°C in less a minute if exposed to room temperature air.

It is strongly recommended that all frozen cell cultures be checked for viability before the stock culture is terminated.

Recycling the CoolCell or COLLCELL LX to room temperature.

The CoolCell is ready to freeze again as soon as the core (black ring) is at room temperature. To rapidly recycle the CoolCell to room temperature, remove the center solid core ring by inverting and tapping on a surface. The CoolCell body and lid will return to room temperature in 10 to 15 minutes. Check that all chambers are dry. Dry the core (black ring) before re-inserting into the central chamber.

Cell freezing performance
The CoolCell will freeze 12 tubes each containing 1ml of cell suspension at -1°C per minute when placed in a -80°C environment (mechanical freezer or dry ice locker). The five consecutive run freezing profile curves at right were performed with 12 sample loads each.



Further products of interest:
Cryopreservation media:
CryoStor 10 – Cat#:PB-210102 (10% DMSO containing medium)
CryoNovo P24 – Cat#:PB-AK9932 (DMSO-free medium)

For further information please do not hesitate to contact us by phone under: +49 (0)89 517 286 59-0 or by Email: info@pelobiotech.com.