

# Technical Notes—How to Passage and Subculture—HUVEC

## General Media Requirements

HUVEC can achieve 15 population doublings with AllCells HUVEC Basal Medium (HUVEC-002) and AllCells HUVEC Basal Medium Supplement (HUVEC-003).

## Thawing and Culturing Cells

1. Calculate the number of flasks needed. The recommended seeding density for AllCells HUVEC is 2,500 to 5,000 cells/cm<sup>2</sup>. For example, one vial of AllCells HUVEC (5x10<sup>5</sup>/vial) is sufficient for four to eight T25 flasks.
2. Prepare the flasks and the culture media. Add 2ml of 0.5% Gelatine to each T25 flask or 5ml 0.5% of Gelatine to each T75 flask. At 37°C, incubate for >2 hours or overnight. After the incubation, discard the Gelatine and wash the flask once with 10ml DPBS. To the 90ml of AllCells HUVEC Basal Media (HUVEC-002) Supplement, add the entire contents of 10ml AllCells HUVEC Basal Medium Supplement (HUVEC-003). Add 1 ml/5 cm<sup>2</sup> of the mixed media to the Gelatine-coated flasks. In a 37°C, 5% CO<sub>2</sub> humidified incubator, allow the flasks to equilibrate for at least 30 minutes.
3. Wipe the frozen vial with 70% alcohol before thawing. In a biosafety hood, briefly twist the cap a quarter-turn to relieve pressure, then retighten the cap.
4. In a 37°C water bath, quickly thaw the vial. Be careful not to submerge the entire vial in the water bath. Do not remove the vial until a tiny ice-crystal is left. Wipe the outside of the vial with 70% alcohol.
5. Resuspend and dispense cells from the vial into the culture flasks set up earlier. Gently rock the culture flasks to evenly distribute the cells and return to the incubator.

**Note:** It is not necessary to wash the cells. Washing the cells is more damaging than the effects of DMSO residue in the culture.

## Maintaining Cell Culture

1. Change the growth medium the day after seeding and every other day thereafter.
2. In a sterile container, warm an appropriate amount of medium to 37°C. Remove the medium and replace it with the warmed, fresh medium. Return the flask to the incubator.

**Note:** Avoid repeated warming and cooling of the medium. If the entire contents are not needed for a single procedure, transfer only the required volume to a sterile secondary container.

## Subculturing Cells

1. Subculture the cells when they are 70 to 80% confluent (about seven days). Many mitotic figures should be visible throughout the flask.

2. For each 25cm<sup>2</sup> of cells to be subcultured:
  - a. Prepare 2 ml of Trypsin/EDTA, 5 ml of HEPES Buffered Saline Solution (HEPES-BSS), 4 ml of Trypsin Neutralizing Solution (TNS such as 10% FBS-DPBS). Allow the aforementioned items to come to room temperature.
  - b. From one culture flask, remove the medium and rinse the flask with 5 ml of room temperature HEPES-BSS. (This is an IMPORTANT step. The culture medium contains complex proteins that neutralize the effect of trypsin.) Remove the HEPES-BSS solution from the flask;
  - c. Repeat **Step b** for each culture flask.
3. Add 2 ml of Trypsin/EDTA solution to each T25 flask. Allow the trypsinization to continue until approximately 90% of the cells are have lifted up from the T25 flask. This entire process takes about two to six minutes. Hit the flask against the palm of your hand to release the majority of cells from the culture surface. If only a few cells detach, wait 30 seconds and repeat this motion again. If cells still do not detach, wait and repeat every 30 seconds thereafter.
4. After cells have detached, neutralize the trypsin in the flask with 4 ml of room temperature Trypsin Neutralizing Solution. (If the majority of cells do not detach within 7 minutes, the trypsin is either not warm enough or not active enough to release the cells. Harvest the culture vessel as described above and either re-trypsinize with fresh, warm Trypsin/EDTA solution or rinse with Trypsin Neutralizing Solution. Add fresh, warm medium to the culture vessel and return to an incubator until fresh trypsinization reagents are available.)
5. Quickly transfer the detached cells to a sterile 15ml centrifuge tube and rinse the flask with 2 ml of HEPES-BSS to collect residual cells. Add this rinse to the centrifuge tube.
6. Under the microscope, examine the harvested flask to make sure the harvest was successful. Look at the number of cells left behind. This number should be less than 5%.
7. Centrifuge the harvested cells at 220 x g for 5 minutes to pellet the cells.
8. Dilute the cells in 4 to 5 ml of growth medium and count the cells with a hemacytometer.
9. Culture the cells (Refer to the Thawing and Culturing Cells section).
10. Warm an appropriate amount of medium to 37°C.