Rev. 1

# 3D Endothelial–Pericyte Coculturing Kit

(3D-EPC)

Catalog #8728

## Product Description

Blood vessels are responsible for transporting blood throughout the body as part of the circulatory system. Their main components are endothelial cells, which comprise the inner lining, and mural (or perivascular) cells, which are largely responsible for regulating vasoconstriction, pruning, permeability, and vascular maturation. There are two main types of mural cells: vascular smooth muscle and pericytes. Pericytes wrap around capillary vessels and communicate with other cells using their long cytoplasmic processes. Vessels that lack or have lost pericyte investment are shown to become hemorrhagic or hyperdilated. Unlike vascular smooth muscle cells, pericytes have roles beyond scaffolding evidenced by their direct communication and physical contact with endothelial cells, and their density differs with respect to their function and capillary bed. Among their many functions, pericytes most notably contribute to blood brain barrier functionality and their loss has been found to contribute to neurodegenerative diseases and stroke. ScienCell's 3D Endothelial-Pericyte Coculturing Kit (3D-EPC) combines both pericytes and endothelial cells in one 3-dimensional *in vitro* assay that utilizes a defined serum-free medium to mimic the vasculature more closely in a controlled environment (see Figure 1 at end of protocol).

**Kit Components**

| Cat # | # of vials | Name | Quantity | Storage |
|---|---|---|---|---|
| 8728-a | 1 | Collagen I from rat tail, 4 mg/mL | 10 mL | 2-8 °C |
| 8728-b | 1 | Buffer A, 10X | 1.5 mL | 2-8 °C |
| 8728-c | 1 | Buffer B | 1 mL | 2-8 °C |
| 8728-d | 1 | sterile $H_2O$ | 5 mL | 2-8 °C |
| 8000 | 1 | Human Umbilical Vein Endothelial Cells (HUVEC) | 5 x $10^5$ | liquid nitrogen |
| 1001-b | 1 | Endothelial Cell Medium - basal | 500 mL | 2-8 °C |
| 1052 | 1 | Endothelial Cell Growth Supplement | 5 mL | -20°C |
| 0025 | 1 | Fetal Bovine Serum | 25 mL | -20°C |
| 0503 | 1 | Penicillin/streptomycin Solution | 5 mL | -20°C |
| 1200-3D | 1 | Human Brain Vascular Pericytes (HBVP-3D) | 1 x $10^5$ | liquid nitrogen |
| 1201-3D | 1 | Pericyte Medium – 3D | 100 mL | 2-8 °C |
| 1252-3D | 1 | Pericyte Growth Supplement – 3D | 1 mL | -20°C |
| 0002-3D | 1 | Fetal Bovine Serum | 2 mL | -20°C |
| 8001 | 2 | 3D Medium - basal - serum free | 100 mL | 2-8 °C |
| 8052 | 2 | 3D Growth Supplement | 1 mL | -20°C |
| 0573 | 3 | Penicillin/streptomycin Solution | 1 mL | -20°C |

**Additional Materials Recommended (Not Included)**

| Cat # | Product Name |
|---|---|
| 0183 | 0.05% Trypsin/EDTA (T/E) |
| 0113 | Trypsin Neutralization Solution (TNS) |
| 0303 | Dulbecco's Phosphate-Buffered Saline (DPBS) |
| 8248 | Bovine Plasma Fibronectin |
| 0413 | Poly-L-Lysine, 10 mg/mL |

**Quality Control**
3D-EPC is tested for the formation of lumen-containing HUVEC tubules according to the included protocol. All components are negative for bacterial and fungal contamination.

**Product Use**
3D-EPC is for research use only. It is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.

**Shipping**
8728-a, 8728-b, 8728-c, 8728-d are shipped on gel ice; 1001-b, 8001, and 1201-3D are shipped at room temperature; 8000, 1200-3D, 1052, 0025, 0503, 1252-3D, 0002-3D, 8052, and 0573 are shipped on dry ice.

**Procedure:**

**Important notes before starting:**
- We recommend making about 500 µL of extra gel to account for gel lost during pipetting.
- Keep all kit components on ice until ready to use. Gel polymerization is affected by temperature.
- We do not recommend extensive sub-culturing of cells prior to performing 3D assays. Sub-culturing can select for 2D growing characteristics, which can affect 3D assaying efficacy.

**A. Initiating HUVEC**
1. Thaw HUVEC (Cat# 8000) vial following the instructions detailed in the product sheet for Cat# 8000.
2. If more cells are required, cells can be subcultured following the instructions detailed in the product sheet for Cat# 8000.
3. Determine how many cells are needed for your experiments and calculate accordingly.
    a. Please note that each well or gel dot requires about $7.5x10^4$ viable HUVEC; scale appropriately.

**B. Initiating HBVP**
1. Thaw HBVP-3D (Cat# 1200-3D) vial following the instructions detailed in the product sheet for Cat# 1200. Use 1 T-25 flask for culturing the cells.
2. If more cells are required, cells can be subcultured following the instructions detailed in the product sheet for Cat# 1200.
3. Determine how many cells are needed for your experiments and calculate accordingly.
    a. Please note that each well or gel dot requires about $1.5x10^4$ viable HBVP; scale appropriately.

**C. Preparation of 3D gel dots: preparation time ~1.25 hour, designed for 24-well plates.** *Protocol details instructions to make 1 mL of collagen gel. Each "gel dot" requires 75 µL of gel per well. Please scale appropriately.*

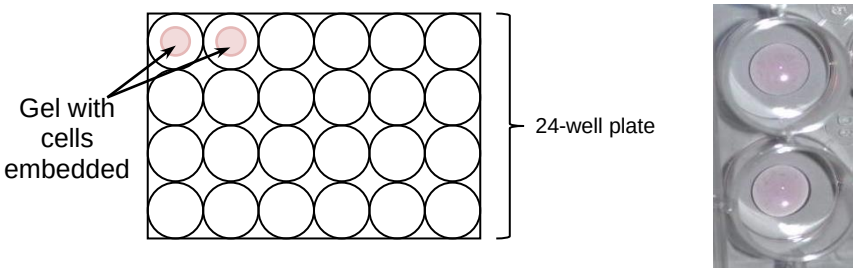
1. When desired amount of HUVEC and HBVP have been achieved from Sections A and B, gather the necessary materials to prepare 3D assay: Cat# 8728-a, Cat# 8728-b, Cat# 8728-c, Cat# 8728-d, Cat# 8001, Cat# 8052, Cat# 0573, $7.5x10^4$ HUVEC per intended gel dot (from Section A), $1.5x10^4$ HBVP per intended gel dot (from Section B), 0.05% Trypsin/EDTA, DPBS, and a Trypsin Neutralization solution, and ice.
2. To prepare complete 3D medium-serum free by adding 1 mL 3D Growth Supplement (Cat# 8052) and 1 mL pen/strep (Cat# 0573) to 100 mL of Cat# 8001.
   a. Store prepared medium at 2-8°C when not in use; use at room temperature with assay.
3. Harvest HUVEC and HBVP from culturing vessels following instructions provided in Cat# 8000 and Cat# 1200.
4. Once cells have been harvested and counted, aliquot $7.5x10^4$ HUVEC for each intended embedded gel dot in an appropriately sized tube.
5. To each of those HUVEC-containing tube(s) from Step C4, add $1.5x10^4$ HBVP for each intended embedded gel dot.
   a. Example 1: For 1 75- µL gel dot, aliquot $7.5x10^4$ HUVEC and $1.5x10^4$ HBVP into the same tube.
   b. Example 2: For 5 75-µL gel dots, aliquot $3.75x10^5$ HUVEC and $7.5x10^4$ HBVP into the same tube.
   c. Note: The ratio of HBVP to HUVEC must be maintained. HBVP density affects tubule formation and gel rigidity.
6. Centrifuge the cells at 1,100 rpm (2 rcf) for 5 minutes and set aside on ice.
   a. Leave the excess media with the cell pellet to remove just prior to collagen addition before plating.
7. Obtain an uncoated 24-well plate for plating. Prepare gel components in a separate tube by combining the following components listed in the table below. Example shown is for 1 mL collagen gel; please scale appropriately. Maintain all components on ice during this step.

Making Collagen Gels (for 1 mL gel)

| Component | Volume |
|---|---|
| Collagen I (Cat#8728-a) | 625 µL |
| Buffer A (Cat#8728-b) | 100 µL |
| Sterile $H_2O$ (Cat#8728-d) | 225µL |

   a. The final proportions of HUVEC/HVBP/gel should be $7.5x10^4$ HUVEC/$1.5x10^4$ HBVP/75 µL gel for each 75- µL gel dot.
8. Mix contents well with gentle pipetting after adding each reagent and avoid bubbles.
9. Retrieve pelleted cells and remove excess medium.
10. To collagen mixture from Step 8, add 50 µL Buffer B (Cat# 8728-c). BE QUICK. Gel starts to polymerize with addition of Buffer B. Mix well with gentle pipetting and avoid bubbles.
11. Add the appropriate amount of gel to cells to obtain a final ratio of $7.5x10^4$ HUVEC/$1.5x10^4$ HBVP/75 µL gel. Mix well by pipetting; avoid bubbles.

12. Carefully pipette 75 µL of the cells/gel mixture from Step 11 to the middle of 1 well of a 24-well plate.
13. Once gel dots have been plated, do not tilt or move plate in hood for 5 minutes. Doing so may cause gel to spread to the well edge and disrupt tubule formation.
14. The gel dots with embedded cells will approximate this diagram (left) and photo of gel dots properly plated (right):






Top view

15. Carefully place the plate in a 37 °C/5% $CO_2$ humidity incubator and let the gel polymerize undisturbed for 1-2 hours.
16. After polymerization, gently add 1 mL room temperature complete 3D Medium from Step 2 to each gel dot well drop-wise and down the side of the well.
    a. Aggressive addition of media can dislodge the gel dot.
    b. Cold media can disrupt the integrity of the gel.
17. Maintain in a 37°C/5% $CO_2$ humidity incubator and change the medium every other day.
    a. Remove media using a pipette, as vacuum aspiration can dislodge the gel.
18. Observe cells daily; assay typically peaks around days 7-9 but can be maintained past day 9. See Figure 1 below for tubule formation. Gels can be fixed and stained for quantitative assessment using 3D Gel Staining Kit (Cat# 8808).

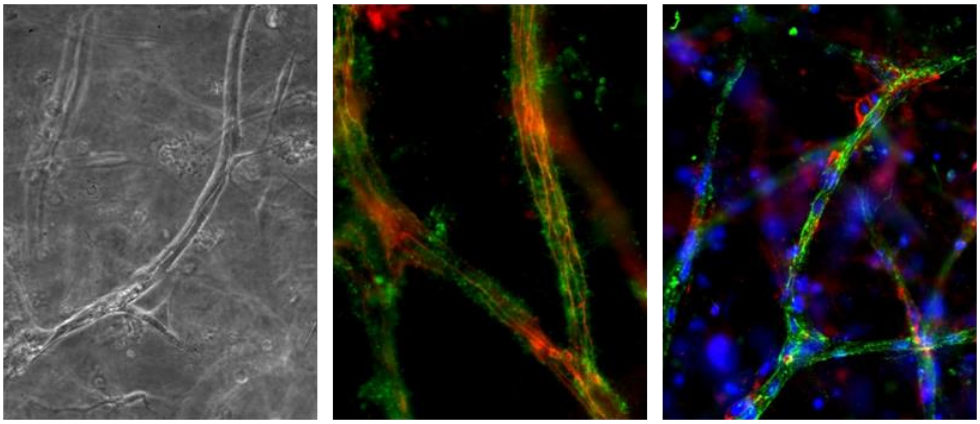


Figure 1. (Left) Phase contrast image of lumen-containing tubule, day 9, 200x. (Center) Fluorescence image of multicellular tubule showing endothelial marker CD31 staining in green and tight junction protein ZO-1 staining in red, day 9, 400x. (Right) Fluorescence image of multicellular tubule showing endothelial marker VWF staining in green, pericyte marker NG-2 staining in red, and DAPI staining in blue, day 9, 200x.