Rev. 1

3D Embedded Tubule Formation Kit

(3D-ETF)

Catalog #8708

Product Description

Angiogenesis is the formation of new blood vessels from preexisting vessels. Angiogenesis research is relevant in numerous contexts such as organ development, tissue repair, wound healing, and tumor progression. Angiogenesis is a complex physiological process that involves cell survival, proliferation, migration, extracellular matrix degradation, altering cell-cell adhesion, cellular differentiation, network formation, lumen formation, and pruning. Due to the complexities, angiogenesis is difficult to study in a 2-dimensional *in vitro* system, which inherently lacks multiple aspects of the physiological angiogenic microenvironment. ScienCell™'s 3-dimensional Embedded Tubule Formation Kit (3D-ETF) is an inclusive kit that utilizes purified collagen type I to mimic all the intricacies of the angiogenic process more closely, including the lumen formation step.

Kit Components

Cat #	# of vials	Name	Quantity	Storage
8708-a	1	Collagen I from rat tail, 4 mg/mL	10 mL	2-8 °C
8708-b	1	Buffer A, 10X	1.5 mL	2-8 °C
8708-c	1	Buffer B	1 mL	2-8 °C
8708-d	1	Sterile H_2O	5 mL	2-8 °C
8000	1	Human Umbilical Vein Endothelial Cells (HUVEC)	5×10^5	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
8001	2	3D Medium - basal - serum free	100 mL	2-8 °C
8052	2	3D Growth Supplement	1 mL	-20°C
0573	2	Penicillin/Streptomycin Solution	1 mL	-20°C

Additional Recommended Materials (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
N/A	Sterile 24-well plate

Quality Control

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

Shipping
8708-a, 8708-b, 8708-c, 8708-d are shipped on gel ice; 1001-b and 8001 are shipped at room temperature; 8000, 1052, 0025, 0503, 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 cells; scale appropriately.

B. Preparation of 3D gel dots: preparation time ~1.25 hr, designed for 24-well plates.

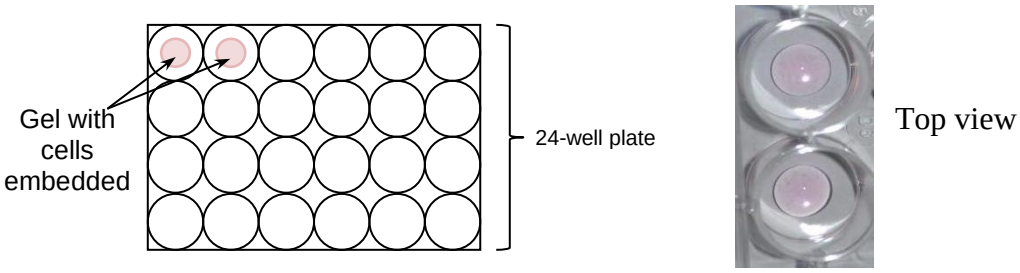
Protocol details instructions to make 1 mL of collagen gel. Each "gel dot" requires 75 µL of gel per well. Please scale appropriately.

1. When desired amount of HUVEC have been achieved from Section A, gather the necessary materials to prepare 3D assay: Cat# 8708-a, Cat# 8708-b, Cat# 8708-c, Cat# 8708-d, Cat# 8001, Cat# 8052, Cat# 0573, $7.5x10^4$ HUVEC per intended gel dot (from Section A), 0.05% Trypsin/EDTA, DPBS, Trypsin Neutralization Solution, and ice.
2. 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 bottle of Cat# 8001.
    a. Store prepared medium at 2-8°C when not in use; use at room temperature with assay.
3. Harvest HUVEC from culturing vessel following instructions provided in Cat# 8000.
4. Once cells have been harvested and counted, aliquot $7.5x10^4$ cells for each intended embedded gel dot in an appropriately sized tube.
5. 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 and remove just prior to collagen addition before plating.
6. Obtain an uncoated 24-well plate(s) 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#8708-a)	625 µL
Buffer A (Cat#8708-b)	100 µL
Sterile H_2O (Cat#8708-d)	225 µL

7. Mix contents well with gentle pipetting after adding each reagent and avoid bubbles.
8. Retrieve pelleted cells and remove excess medium.
9. To the collagen mixture from Step 7, add 50 µL Buffer B (Cat# 8708-c). BE QUICK. Gel starts to polymerize with addition of Buffer B. Mix well with gentle pipetting and avoid bubbles.
10. Add the appropriate amount of gel to cells to obtain a final ratio of $7.5x10^4$ cells/75 µL gel. Mix well by pipetting; avoid bubbles.
11. Carefully pipette 75 µL of cell/gel mixture from Step 10 to middle of 1 well of a 24-well plate.
12. 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.
13. The gel dots with embedded cells will approximate this diagram (left) and photos of gel dots properly plated from the top (middle) and viewed from the side (right):



14. Carefully place the plate in a 37°C/5% CO_2 humidity incubator and let the gel polymerize undisturbed for 1-2 hours.
15. 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.
16. 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.
17. Observe cells daily; assay typically peaks around day 6. See Figure 1 below for tubule formation. Gels can be fixed and stained for quantitative assessment using 3D Gel Staining Kit (Cat# 8808).

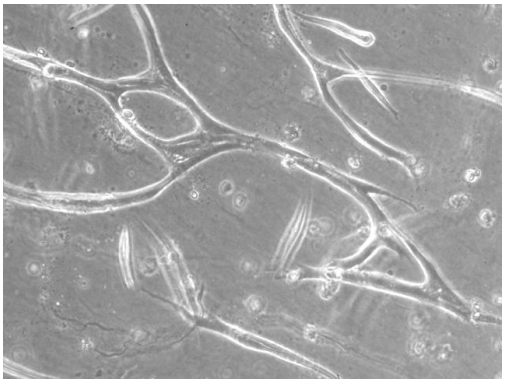


Figure 1. Day 5; phase contrast image of lumen-containing HUVEC tubule, 200X.