Rev. 0

ScienCell™ Research Laboratories

3D-Angiogenesis Assay
3D-AA

Cat. #3D-8000

Product Description

Angiogenesis is the intricate process of the formation of new capillaries from a pre-existing vascular network. Angiogenesis occurs physiologically in situations such as wound healing and muscle growth. It also plays a role in pathophysiological conditions such as in cancer progression. In cancer, a pro-angiogenic state is triggered, resulting in malignant tumor progression [1]. The new vessel formation supports the propagation of the growing tumor by delivering nutrients and oxygen, while at the same time facilitating the removal of metabolic wastes. In ischemic heart disease, angiogenesis has been perceived as a potential therapeutic approach for treating heart failure arising from blood flow deprivation [2]. Given its significance in various conditions, proper tools to study angiogenesis is crucial. ScienCell has developed a ready-to-use 3D-Angiogenesis Assay (3D-AA) that utilizes endothelial cell spheroids that can be readily embedded into the included collagen matrix. Upon successful stimulation, endothelial cells will begin to invade into its surroundings, mimicking the angiogenic process *in vivo*. The better physiological reflection of angiogenesis serves as an advantage of the 3D-AA over other *in vitro* assays such as the tube formation assay. 3D-AA is an excellent assay to conveniently and rapidly screen test compounds for their pro-angiogenic and anti-angiogenic effects. Within 24 to 48 hours, the number and length of sprouts formed can be quantified and utilized as a measure of the pro- or anti-angiogenic effect of the test substance.

Kit Components (Included)

3D Cell Culture Components				
Cat #	**# of vials**	**Product Name**	**Quantity**	**Storage**
SP-8000	1	Human Umbilical Vein Endothelial Cell Spheroids (SP-HUVECS)	4×10^3 spheroids	Liquid nitrogen
3D-1001	1	3D-Endothelial Cell Spheroid Medium (3D-ECSpM)	200 mL	2-8 °C
0040	1	Fetal Bovine Serum (FBS)	40 mL	-20 °C
0583	1	Penicillin/Streptomycin Solution (P/S)	2 mL	-20 °C
3D-8000-a	2	Collagen I from rat tail, 4 mg/mL	10 mL	2-8 °C
3D-8000-b	2	Buffer A, 10X	1.5 mL	2-8 °C
3D-8000-c	2	Buffer B	1 mL	2-8 °C
3D-8000-d	2	Sterile H_2O	5 mL	2-8 °C
8001	1	3D Medium – basal – serum free	100 mL	2-8 °C
0573	1	Penicillin/streptomycin Solution	1 mL	-20 °C
0343 (or) 0353 (or) 0383	1	Ultra-Low Binding Culture Plates (24-, 48-, or 96- well plate)	1 plate	RT

3D-8000-e	1	Vascular Endothelial Growth Factor, 1 ug/mL (VEGF)	1 mL	-20 °C

Quality Control
3D-AA is tested for the formation of HUVEC spheroid sprouts induced by VEGF according to the included protocol. All components are negative for bacterial and fungal contamination.

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

Shipping
SP-8000, 0020, 0583, 0573 and 3D-8000-e are shipped on dry ice. 3D-1001, 3D-8000-a, 3D-8000-b, 3D-8000-c, 3D-8000-d, 8001, and (0343 or 0353 or 0383) are shipped at room temperature.

References
[1] Baeriswyl V, Christofori G. (2009) "The angiogenic switch in carcinogenesis." *Semin Cancer Biol*. 19: 329-337.
[2] Johnson T, Zhao L, Manuel G, Taylor H and Liu D. (2019) "Approaches to Therapeutic Angiogenesis for Ischemic Heart Disease." *J Mol Med*. 97 (2): 141-151.

Procedure:

<u>Step I: Preparing the complete 3D culture medium</u>

1. Thaw fetal bovine serum (FBS; Cat. #0040), and penicillin/streptomycin solution (P/S solution; Cat. #0583) at 37°C. Mix FBS, and P/S solution into the 3D-endothelial cell spheroid medium (3D-ECSpM; Cat. #3D-1001) by gently swirling the medium bottle around.

    a. 3D-ECSpM is **viscous** and optimized for homogenous spheroid formation.
    b. Warm the complete 3D-ECSpM to **room temperature** before use.
    c. When stored in the dark at 4°C, the complete medium is stable for one month.

<u>Step II: Thawing the ready-to-use 3D spheroids</u>

2. One frozen vial contains $\geq 4 \times 10^3$ spheroids, which is sufficient for plating into one multi-well plate (e.g. 24-, 48-, and 96-well ultra-low binding culture plate).

3. Place the frozen vial in a 37°C water bath. Hold and rotate the vial gently until the contents completely thaw. Promptly remove the vial from the water bath, wipe it down with 70% ethanol, and transfer it to the sterile field.

4. Carefully remove the cap without touching the interior threads. Gently pipette the spheroid suspension up and down **two times** to disperse potential spheroid aggregates.

5. Gently transfer the spheroid suspension into a fresh 50 mL conical tube.

6. Add 12 mL of 3D culture medium to the above 50 mL conical tube.

7. Resuspend spheroids in 3D culture medium by gently pipetting up and down for ~ 5 times using a serological pipette.

   Note: 3D culture medium has a high viscosity; thus, pipetting slowly is important to avoid bubble formation.

Step III: Preparation of neutralized collagen

8. Prepare on ice: In a 50 mL conical tube, combine 8.125 mL of collagen I (Cat # 3D-8000-a), 1.3 mL of Buffer A (Cat # 3D-8000-b) and 2.925 mL of sterile H_2O (Cat # 3D-8000-d).

9. Add 650 uL of Buffer B (Cat # 3D-8000-c) to the mixture in step 8 and maintain conical tube on ice.
   Note: Neutralized collagen polymerizes rapidly; ensure components and mixtures are placed on ice to prevent premature polymerization. Perform the following procedures quickly but with care.

Step IV: Embedding HUVEC spheroids in collagen

10. Transfer 12 mL of prepared neutralized collagen into spheroid suspension from Step 7 and pipette gently for 5-7 times.

11. Aliquot the suggested volumes (see **Table A, column 2**) of spheroid suspension into each well of the ultra-low binding plate (24-, 48- or 96-well plate) quickly but with care.

Table A: An Example of Suggested Medium Volumes

1	2	3
Plate formats	**Volume per well**	**Expected spheroids per well**
24-well	~ 1000 µL	≥ 150 spheroids
48-well	~ 500 µL	≥ 80 spheroids
96-well	~ 250 µL	≥ 40 spheroids

12. Incubate spheroid mixtures at 37°C in a 5% CO_2 incubator for ≥ 1 hour to allow gel polymerization.

Step V: Preparing test medium

13. Bring 3D medium-basal-serum free (Cat # 8001) to room temperature. Avoid using cold medium - cold medium may perturb gel integrity in later steps.

14. Dissolve test substance in 3D medium-basal-serum free to desired concentration. Each well should be added with 200 µL of assay medium; please scale accordingly if replicates will be performed. If test substance was dissolved in DMSO or alcohol, please ensure that the final DMSO or alcohol content do not exceed 1% in the test medium.

15. Thaw vascular endothelial growth factor (VEGF; Cat. # 3D-8000-e) at 37°C. Combine 500 µL of VEGF and 9.5 mL of 3D medium-basal-serum free in a conical tube to get the final 50 ng/mL concentration of VEGF.

Step VI: Initiating test substance testing

16. Carefully, add 200 µL of the prepared test medium to the respective wells being assayed. Gently release medium to wall of well to prevent dislodging polymerized gels.

17. As a positive control, add 200 µL of 50 ng/mL VEGF to at least one well.

18. Incubate assay plate at 37°C in a 5% CO_2 incubator for 24 - 48 hours.

Step VII: Analyzing the assay

19. Readout of angiogenic activity includes the following parameters:
    a. Number of sprouts
    b. Average sprout length
    c. Cumulative sprout length

20. It is recommended to quantify these parameters in at least 10 spheroids per test condition.

21. Assay can be preserved by adding 1 mL of 4% paraformaldehyde into each well and stored at 4°C.

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Fig. 1 – Brightfield images of the collagen-embedded HUVEC spheroids at 24 hours post VEGF-stimulation.

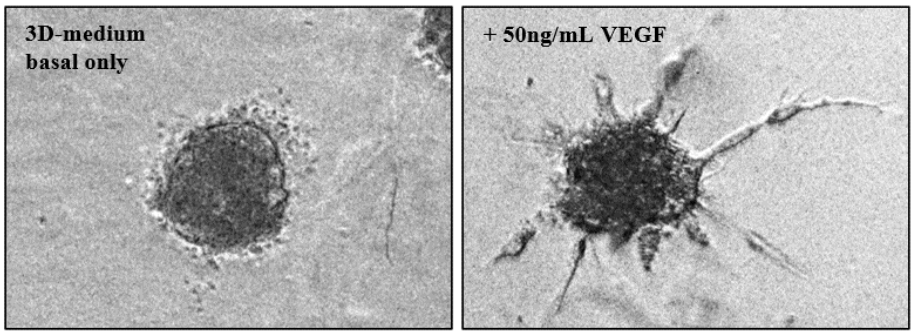

Fig. 2 – Immunofluorescence staining of collagen-embedded HUVEC spheroids at 24 hours post VEGF-stimulation with the endothelial cell marker, von Willebrand Factor (vWF).