Rev. 1

3D Basic Embedded Tubule Formation Kit

(3D-BETF)

Catalog #8698

Product Description

Collagen is a major structural component in the extracellular matrix (ECM) and in connective tissues such as tendons, ligaments, dermis, and blood vessels. As insoluble fibrous proteins, collagens are the primary determinant of ECM tensile strength and they help tissues withstand stretching. While there are at least 28 types of collagen, 80-90% of the collagen in the human body are types I, II, and III. The 3D Basic Embedded Tubule Formation Kit (3D-BETF) contains collagen type I, which is found in skin, tendon, vasculature, and bone ECM, as well as the accessory components needed to form a 3D collagen gel matrix. It also includes a serum-free assay medium optimized to promote endothelial tubule formation within the collagen gels. *Note:* This kit does not include cells. For inclusive kits to create 3-dimensional scaffolds that includes primary cells, see ScienCell™'s 3D Embedded Tubule Formation Kit (Cat. #8708), ScienCell™'s Network Formation Assay Kit (Cat. #8718), and ScienCell™'s Endothelial–Pericyte Coculturing Kit (Cat. #8728).

Kit Components

Cat #	# of vials	Name	Quantity	Storage
8698-a	1	Collagen I from rat tail, 4 mg/mL	10 mL	2-8 °C
8698-b	1	Buffer A, 10X	1.5 mL	2-8 °C
8698-c	1	Buffer B	1 mL	2-8 °C
8698-d	1	sterile H_2O	5 mL	2-8 °C
8001	1	3D Medium - basal - serum free	100 mL	2-8 °C
8052	1	3D Growth Supplement	1 mL	-20°C
0573	1	Penicillin/streptomycin Solution	1 mL	-20°C

Note: This kit does not include cells.

Quality Control

3D-BETF is tested for the formation of lumen-containing HUVEC tubules. All components are negative for bacterial and fungal contamination.

Product Use

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

Shipping

8698-a, 8698-b, 8698-c, 8698-d are shipped on gel ice; 8001 is shipped at room temperature; 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.
- Cells are not included. Count and centrifuge cells to pellet before preparing gel. The optimum number of cells may depend on the cell type and application and should be titrated to determine the optimal density.

Preparation of 3D gel dots: preparation time ~1.25 hours, 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.

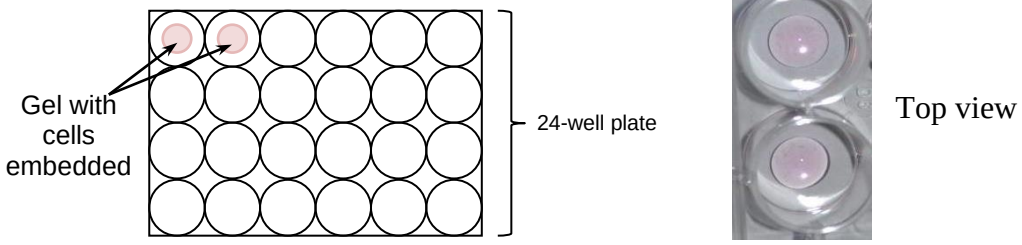
1. Prepare complete 3D Medium - serum free by adding 1 mL 3D Growth Supplement (Cat# 8052) and 1 mL pen/strep (Cat# 0573) to 100 mL bottle of Cat# 8001.
    a. Store prepared medium at 2-8°C when not in use; use at room temperature with assay.
2. Harvest endothelial cells (not included) from culturing vessel.
3. Once cells have been harvested and counted, aliquot cells for each intended embedded gel dot in an appropriately sized tube. For Human Umbilical Vein Endothelial Cells we recommend 7.5×10^4 cells for each embedded gel dot.
4. 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.
5. 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#8698-a)	625 µL
Buffer A (Cat#8698-b)	100 µL
Sterile H_2O (Cat#8698-d)	225 µL

6. Mix contents well with gentle pipetting after adding each reagent and avoid bubbles.
7. Retrieve pelleted cells and remove excess medium.
8. 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.
9. Add the appropriate amount of gel to cells. Mix well by pipetting; avoid bubbles.
10. Carefully pipette 75 µL of cell/gel mixture from Step 13 to middle of 1 well of a 24-well plate.
11. 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.

12. 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):



13. Carefully place the plate in a 37°C/5% CO_2 humidity incubator and let the gel polymerize undisturbed for 1-2 hours.
14. 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.
15. 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.