3D Basic Embedded Tubule Formation Kit

(3D-BETF)
Cat #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	3	3D Medium - basal - serum free	100 mL	2-8 °C
8052	3	3D Growth Supplement	1 mL	-20°C
0573	3	Penicillin/streptomycin Solution	1 mL	-20°C

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: Keep all kit components chilled on ice until ready for use.

- We recommend always making about 500 µL of extra gel to account for gel lost during pipetting.
- Gel polymerization is affected by temperature.
- All work should be performed in a sterile flow hood to maintain sterility; no components should be opened outside of a sterile working environment.
- Decontaminate external surfaces of component receptacles with 70% ethanol prior to entering the sterile working environment.
- Protocol details instructions to make 1 mL of collagen gel; please scale appropriately
  - Each "gel dot" requires 75 µL of gel per well.
  - Please scale appropriately.
- Kit is designed for 75 µL embedding dots in 24-well plates.
- Cells are not included.

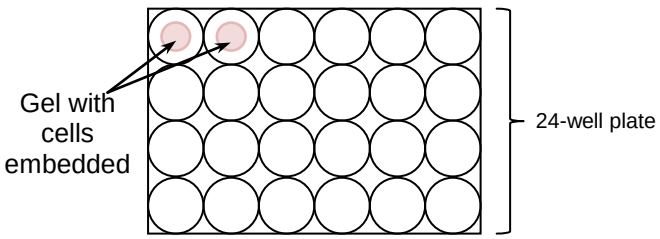
Preparation of 3D gel dots: preparation time ~1.25 hr, designed for 24-well plates

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

1. Prepare 3D Assay Medium - serum free.
   1.1. Add 1 mL 3D Growth Supplement (8052) and 1 mL pen/strep (0573) to 100 mL 3D Assay Medium - basal - serum free (8001).
   1.2. Store prepared medium at 2-8°C when not in use; use at room temperature with assay.
2. Obtain an uncoated 24-well plate(s) for plating and bring it into the hood.
3. Prepare gel components in a separate tube by combining 625 µL collagen I (8708-a), 100 µL Buffer A (8708-b), and 225 µL sterile H_2O (8708-d).
   3.1. Mix contents well with gentle pipetting after adding each reagent and avoid bubbles.
   3.2. If possible, keep everything on ice while combining components.
   3.3. Note: This step makes 1 mL of gel; please scale appropriately.

********AFTER THIS NEXT STEP, BE AS QUICK AS POSSIBLE without sacrificing care********

4. To the mixture from Step B7, add 50 µL Buffer B (8708-c).
   4.1. Mix well with gentle pipetting and avoid bubbles.
   4.2. BE QUICK; gel starts to polymerize immediately with addition of Buffer B.
5. Desired cells may be embedded within collagen gel by pelleting desired number of cells, removing excess media, and adding gel solution from step 4 to cell pellet.
   5.1. Mix well by pipetting; avoid bubbles.
6. Carefully pipette 75 µL of the gel mixture from Step 5 to the middle of 1 well of a 24-well plate.
   6.1. Once gel dots have been plated, avoid tilting or moving the plate.
   6.2. The gel dots will approximate this diagram (left) and photos of gel dots properly plated from the top (right):



7. Leave plate undisturbed in hood for 5 minutes.
8. Carefully place the plate in a 37 °C/5% CO_2 humidity incubator and let the gel polymerize undisturbed for 1 hour.
9. After polymerization, gently add room temperature or warm complete 3D Medium from Step B1.1 to each gel dot well dropwise and down the side of the well.
    9.1. Aggressive addition of media can dislodge the gel dot.
    9.2. Cold media can disrupt the integrity of the gel.
    9.3. Add enough media so that the gel dot is entirely covered (typically around 700 µL).
10. Maintain the assay in a 37 °C/5% CO_2 humidity incubator and change the medium every other day.
    10.1. Do not use a vacuum aspirator as aggressive aspiration can dislodge the gel.
    10.2. Remove media using a pipette by hand.