Rev. 1

3D Collagen I Matrix Kit
(3D-CMK)
Catalog #8688

Introduction
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 Collagen I Matrix Kit (3D-CMK) 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. Note: for inclusive kits to create 3-dimensional scaffolds with 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
The ScienCell™ 3D Collagen I Matrix Kit contains collagen I purified from rat tail tendon, 10X Buffer A, Buffer B, and sterile H_2O.

Cat. #	# of vials	Name	Quantity	Storage
8688-a	1	Collagen I from rat tail, 4mg/ml	25 ml	2-8°C
8688-b	1	Buffer A, 10x	5 ml	2-8°C
8688-c	1	Buffer B	1 ml	2-8°C
8688-d	3	Sterile H_2O	5 ml	2-8°C

Note: See ScienCell™ Cat. #8708 (3D-ETF), Cat. #8718 (3D-NF), and Cat. #8728 (3D-EPC) for complete and inclusive 3D collagen-based kits to assess endothelial network formation and tubule formation.

Quality Control
3D-CMK is tested for gel formation and HUVEC tubule formation. It is negative for bacterial and fungal contamination.

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

Shipping
3D-CMK is shipped on gel ice.

Procedure: Preparation time ~1 hr, designed for 24-well plates

Important notes before starting procedure

- Keep all kit components chilled on ice until ready for use. Gel polymerization is affected by temperature.
- We recommend making about 500 µL of extra gel to account for gel lost during pipetting.
- Protocol is designed to make 1 mL of 2 mg/mL collagen gel.
    - Designed for gels in 24-well plates.
    - Each well requires 300 µL of gel.
        - Gel thickness may be adjusted by adding more or less gel per well.
    - Please scale appropriately.
- Cells and cell culturing media are *not* included in this kit.

1. Prepare gel components by combining 500 µL collagen (Cat. #8688-a), 100 µL Buffer A (Cat. #8688-b), and 388.5 µL sterile H_2O (Cat. #8688-d).
    a. Mix contents well with gentle pipetting; avoid bubbles.
2. Prepare an uncoated, sterile 24-well plate(s) for plating.
3. To the mixture from Step 1, add 11.5 µL Buffer B (Cat. #8688-c).
    a. Mix well with gentle pipetting; avoid bubbles.
    b. BE QUICK; gel starts to polymerize immediately with addition of Buffer B.
4. Pipette 300 µL of gel mixture from step 3 to the middle of each well of a 24-well plate.
5. Carefully place the plate in a 37 °C/5% CO_2 humidity incubator and let the gel polymerize for 1 hour.
6. Submerge polymerized gel with appropriate cell culturing media.
    a. Aggressive addition of media can dislodge the gel.
    b. Cold media can disrupt the integrity of the gel.
    c. Use enough culturing media to cover the entire gel.
7. Maintain gels 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.

To determine the volume of components needed:

- Volume of collagen (Vc) = $\frac{\text{(desired final collagen concentration) x [total desired volume (Vt)]}}{\text{initial concentration of collagen (4 mg/mL)}}$
- Volume of Buffer A (Va) = $\frac{\text{total desired volume (Vt)}}{10}$
- Volume of Buffer B (Vb) = (Vc) x 0.023
- Volume of sterile H_2O (Vh) = (Vt) – (Vc) – (Va) – (Vb)