



**3D Collagen I Matrix Kit**  
(3D-CMK)  
Cat #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<sub>2</sub>O.

<b>Cat. #</b>	<b># of vials</b>	<b>Name</b>	<b>Quantity</b>	<b>Storage</b>
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 <sub>2</sub> O	5 ml	2-8°C

**Note:** See ScienCell™ Cat #8708 (3D-ETF), #8718 (3D-NF), and #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

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### Important notes before starting procedure

- Keep all kit components chilled on ice until ready for use
  - We recommend making about 500  $\mu\text{L}$  of extra gel to account for gel lost during pipetting
  - Gel polymerization is affected by temperature
  - All work is to be done in a sterile flow hood to maintain sterility; surfaces should be decontaminated prior to entry and no components should be opened outside of the sterile area
  - If possible, keep all liquid components on ice throughout procedure until ready for plating
    - Gelling occurs rapidly at room temperature
    - If possible, perform all component manipulations on ice in a sterile flow hood
  - Protocol is designed to make 1 mL of 2 mg/mL collagen gel
    - Designed for gels in 24-well plates
    - Each well requires 300  $\mu\text{L}$  of gel
      - Gel thickness may be adjusted by adding more or less gel per well
    - Please scale appropriately
    - Calculations may be done to adjust collagen concentration in gels
      - Refer to note at end of protocol
  - Cells and cell culturing media are *not* included in this kit
1. Prepare gel components by combining 500  $\mu\text{L}$  collagen (Cat. #8688-a), 100  $\mu\text{L}$  Buffer A (Cat. #8688-b), and 388.5  $\mu\text{L}$  sterile  $\text{H}_2\text{O}$  (8688-d)
    - 1.1. Mix contents well with gentle pipetting; avoid bubbles
  2. Prepare an uncoated, sterile 24-well plate(s) for plating

\*\*\*\*\***AFTER THIS NEXT STEP, action needs to BE AS QUICK AS POSSIBLE without sacrificing care**\*\*\*\*\*

3. To the mixture from Step 1, add 11.5  $\mu\text{L}$  Buffer B (8688-c)
  - 3.1. Mix well with gentle pipetting; avoid bubbles
  - 3.2. **BE QUICK**; gel starts to polymerize immediately with addition of Buffer B
4. Pipette 300  $\mu\text{L}$  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%  $\text{CO}_2$  humidity incubator and let the gel polymerize for 1 hour
6. Submerge polymerized gel with appropriate cell culturing media
  - 6.1. Aggressive addition of media can dislodge the gel
  - 6.2. Cold media can disrupt the integrity of the gel
  - 6.3. Use enough culturing media to cover the entire gel
7. Maintain gels in a 37 °C/5%  $\text{CO}_2$  humidity incubator and change the medium every other day
  - 7.1. Do not use a vacuum aspirator as aggressive aspiration can dislodge the gel
  - 7.2. Remove media using a pipette by hand

Note: To adjust collagen concentration in gels, determine the total volume of collagen needed and then determine the amount of reagents necessary to achieve a normal osmolality and pH.

To determine the volume of components needed:

- Volume of collagen ( $V_c$ ) =  $\frac{\text{desired final collagen concentration} \times [\text{total desired volume (Vt)}]}{\text{initial concentration of collagen (4 mg/mL)}}$

- Volume of Buffer A ( $V_a$ ) =  $\frac{\text{total desired volume } (V_t)}{10}$
- Volume of Buffer B ( $V_b$ ) =  $(V_c) \times 0.023$
- Volume of sterile  $H_2O$  ( $V_h$ ) =  $(V_t) - (V_c) - (V_a) - (V_b)$