Collagen I-3D Gelling Kit
(C3DGK)
Catalog #8178, *100 tests*

Product Description
Type I collagen, a fibrous protein abundant in connective tissues including tendon, ligament, dermis and blood vessel, is the major component and the primary determinant of tensile strength of the extracellular matrix (ECM). It is widely used as a thin layer on tissue-culture surfaces to enhance the attachment and proliferation of a variety of cells including endothelial cells, fibroblasts, hepatocytes, epithelial cells and etc. In addition, collagen I can self-assemble into a 3-D supramolecular gel *in vitro*, making it an ideal biological scaffold to promote more *in vivo*-like cellular morphology and function.

The ScienCell™ collagen I-3D Gelling kit includes collagen I purified from rat tail tendon [1] and supplied as a sterile liquid in 1/1000 acetic acid. It also includes a 10× Gelling Buffer, which can be used to adjust the pH and ionic strength of the collagen I solution to allow for the formation of a homogenous gel.

Kit Components

Cat. #	# of vials	Reagent	Quantity	Storage
8178a	1	Collagen I from rat tail (4 mg/ml)	25 ml	2-8°C
8178b	1	Gelling Buffer, 10×	5 ml	2-8°C
8178c	1	Buffer B	1 ml	2-8°C
8178d	1	Sterile H_2O	5 ml	2-8°C

Quality Control
The ScienCell™ collagen I-3D Gelling kit is tested for the formation of collagen gel using primary cells and tested negative for bacterial contamination.

Product Use
C3DGK is used to form a collagen I gel *in vitro* and is for research use only. It is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.

Shipping
All components are shipped on gel ice.

References
[1] Bell E, Ivarsson B, Merrill C. (1979) "Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro." *Proc Natl Acad Sci USA*, 76(3): 1274-1278.

Procedure

A. Preparation of collagen I gel without cells

1. Keep the following items on ice: Type I Collagen (8178a), 10x Gelling Buffer (8178b), and Buffer B (8178c).
2. Determine the volume of Type I Collagen and 10x Gelling Buffer needed as shown below.

$$\text{Volume of Collagen needed} = \frac{(\text{Final Conc. of Collagen}) \times (\text{Total Volume})}{(\text{Initial Conc. of Collagen})}$$

$$\text{Volume of 10x Gelling Buffer needed} = \frac{(\text{Total Volume})}{10}$$

3. On ice, mix Type I Collagen, 10x Gelling Buffer, and sterile H_2O in a sterile tube. As the LAST component, add Buffer B (approximately 1-3 µl per ml of mixture) to bring the pH to the optimal range of 7.0 – 7.5.
4. Add appropriate volume of the mixture (e.g. 100 µl per/cm^2) into desired culture vessels (e.g. tissue culture inserts).
5. Incubate for 30 minutes at 37°C until a homogenous gel is formed. Different collagen I concentration gives matrices of collagen fibrils with different microstructures and mechanical properties. We recommend using 2-4 mg/ml collagen I.
6. Aspirate the expelled solution and rinse with PBS or culture medium before seeding of cells.

B. Preparation of collagen I gel embedded with cell

1. Determine the total cell number or cell seeding density needed for your experiment.
2. Collect the cell pellets with the appropriate cell number for your experiment.
3. Next, follow the above procedures A1-A2.
4. On ice, mix the Type I Collagen, 10x Gelling Buffer, sterile H_2O and cell pellets in a sterile tube. Pipette ~10-15 times to resuspend the cell pellets in Type I Collagen mixture. As the LAST component, add Buffer B (approximately 1-3 µl per ml of mixture) to bring the pH to the optimal range of 7.0 – 7.5.
5. Add appropriate volume of the mixture (e.g. 100 µl per/cm^2) into desired culture vessels (e.g. tissue culture inserts).
6. Incubate for 30 minutes at 37°C until a homogenous gel is formed.
7. Aspirate the expelled solution and add desired culture medium to the collagen I gel embedded with cells. Culture at 37°C in a 5% CO_2 incubator.