Collagen I-3D Gelling Kit
Cat. No. 8178

Introduction

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 extracelluar 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 superamolecular 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 by a modification of the method of Bell *et al*[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. No.	# of vials	Reagent	Quantity	Storage
8178a	1	Collagen I from rat tail	100 mg	2-8°C
8178b	1	Gelling Buffer, 10×	5 ml	2-8°C

Quality Control

The ScienCell™ collagen I-3D Gelling kit is tested for the preparation of dermal equivalents populated with neonatal human dermal fibroblasts and tested and found negative for bacterial contamination.

3D Gel Preparation Procedures

A. Preparation of collagen I gel without cells:

1. Mix 1 part of 10× Gelling Buffer and 9 parts of collagen I solution at 2-8°C, gently titrate in a few drops of sterile 2 N NaOH (approximately 1-3 µl per ml of mixture) to bring the pH to the optimal range of 6.5-7.5. Add appropriate volume of the mixture (e.g. 100 µl per 1 cm^2) into desired culture vessels (e.g. tissue culture inserts). Since the neutralized collagen I solution solidifies quickly at room temperature, we suggest use pre-cooled pipettes and plates. Keep everything cool and handle quickly and gently to avoid bubbles and clumps.
2. 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. Further dilution of collagen I can be obtained by adding 1/1000 acetic acid.
3. Aspirate the expelled solution and rinse with PBS or culture medium before seeding of cells.

B. Preparation of collagen I gel embedded with cells:

1. Collect cell pellets, wash once with PBS and re-suspend cells in fetal bovine serum (FBS) at 10× their final desired concentration (e.g. for the preparation of a dermal equivalent, suspend the

fibroblasts in FBS at 1-5×10^6/ml to give a final concentration of 1-5×10^5/ml in the gel), keep the cell suspension at 2-8°C.

2. Mix 1 part of 10× Gelling Buffer, 1 part of cells suspension and 8 parts of collagen I solution of desired concentration at 2-8°C, gently titrate in a few drops of sterile 2 N NaOH to bring the pH to the optimal range of 6.5-7.5. Add appropriate volume of the mixture (e.g. 100 µl per 1 cm^2) into desired culture vessels (e.g. tissue culture inserts). Keep everything cool and handle with care to avoid bubbles and clumps.
3. Incubate for 30 minutes at 37˚C until a homogenous gel is formed.
4. Aspirate the expelled solution and add desired culture medium to the collagen I gel embedded with cells. Culture in a CO_2 incubator.

References:

1. Bell, E., Ivarsson B. and Merrill C., *Proc. Natl. Acad. Sci. USA*, 76(3), 1274-1278 (1979).