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Protocol

Collagen Solution for Tissue Engineering

I. Compatible Products

Product	Item No.
UltraPure collagen I for tissue engineering, bovine placenta	001-G70-MF1
UltraPure collagen III for tissue engineering, bovine placenta	001-G70-MF3
Collagen I for tissue engineering, bovine placenta	001-G70-MF5
Collagen I for tissue engineering, bovine skin	001-G70-MF7
UltraPure collagen I for tissue engineering, human placenta	012-G70-MF1

II. Additional Supplies

1. Collagen: protein concentration is around 2 mg/mL
2. 5X cell culture medium (RPMI, M199 or DMEM)
3. 10X PBS (sterile filtered)
4. Neutralization Solution (0.1 M NaOH, sterile filtered)

III. Procedure for Gel Solution Preparation

Note: Keep all solutions on ice during the solution preparation.

1. According to Table 1, prepare the desired volume of collagen gel solution in the following sequence.

Table 1. Preparation of collagen gel solution

Total Volume (mL)	Collagen Solution (mL)	Medium (mL)	Neutralization Solution (µL)
10	8	2	250
5	4	1	125
2.5	2	0.5	62.5
1	0.8	0.2	25
0.75	0.6	0.15	18.75
0.5	0.4	0.1	12.5

Note: 5X medium should be compatible with the desired cell type.

   a. In a sterile tube, add the appropriate volume of collagen solution.

   b. Next, add the corresponding volume of 5X PBS or medium. Mix well.
   Note: Solution should be yellow in color.

c. Finally, add the Neutralization Solution and immediately mix well.
Note: Solution should change to pink/red color.

2. After mixing, keep the solution on ice. The pH of the collagen gel solution should be neutral, which is indicated by the pink/red color of phenol red in the 5X medium.

IV. Procedure for Top Culture

1. Harvest and resuspend desired cell line at 0.1 to 2.0 x 106 cells/mL.
2. Pipette a proper size of a chilled collagen gel solution onto a tissue culture plate or dish. See Table 2 for recommended measurements.

Culture Dish	Volume (mL)
96-well	0.1
48-well	0.2
24-well	0.5
6-well	1
3-mm	2
60-mm	3
100-mm	5

3. Immediately transfer to 37°C incubator for 60 minutes to initiate polymerization of the collagen.
Note: The polymerized gel will look cloudy.
4. After formation of the collagen gel, seed desired cells onto the collagen gel.
5. Overlay polymerized collagen gel with culture media.
6. Incubate cells overnight or several days at 37°C with appropriate CO_2 levels. Change medium daily.
7. Cells can be visualized using phase contrast microscopy and can be analyzed by other tests such as cell viability assay.