

Fabrication of Collagen I Gels

1. General Information

This Application Note is a protocol for fabricating collagen I gels with different cell culture media. Depending on the medium's original acidity, the pH of the mixture has to be adjusted to pH=7.4 using sodium hydroxide (NaOH) and sodium bicarbonate (NaHCO₃). The resulting gel will have different concentrations of collagen I, ranging from 1.5 to 0.5 mg/ml. This protocol is designed specifically for the given solutions. For example, utilizing a collagen gel from a different manufacturer may influence the whole composition.

2. Material:

- Collagen I, bovine, 3 mg/ml (PureCol®, Advanced BioMatrix, 5005-B)
- Media:
 - 10 × MEM (Sigma, M0275)
 - 10 × DMEM (c.c.pro, FM-59-L)
 - 10 × Endothelial Cell BM (PromoCell, C-97026, customer formulation)
 - RPMI 1640 (Gibco, 11879)
 - DMEM (c.c.pro, FM-58-L)
 - Endothelial Cell Growth Medium (PromoCell, C-22010)
- NaOH in ultrapure H₂O 1M
- NaHCO₃ 7.5 % (Sigma, S8761)
- L-glutamine 100 × (c.c.pro, Z-10-M)
- Sterile ultrapure water

3. Fabrication Protocol

- The ingredients are listed in the order of pipetting.
- Place all solutions at room temperature half an hour before starting the experiment.
- All 10 × media are without supplements, therefore 10 × DMEM needs to be complemented with L-glutamine before usage.
- For addition of supplements (e.g., growth factors, inhibitors, serums etc.) add the supplements to the 1 × medium (50 µL) without cells.
For example: Prepare a 10x concentration of the supplement in 30 µl of 1x PBS or 1x medium and then add 20 µl of 1x medium.
- Fill the gel into the culture vessel within 5 minutes of assembling all components.
- For gelation, place the gel in a cell culture incubator (37°C, 5 % CO₂) for 45 minutes.
The cells will continue to settle for the first few minutes. Therefore, to avoid the cells settling on the bottom of the vessel, it may be best to incline the chamber vertically (note, this is only possible with chemotaxis chambers and channel slides).

MEM/RPMI 1640				DMEM				EC-Medium			
Collagen (mg/ml)	1.5	1	0.5	Collagen (mg/ml)	1.5	1	0.5	Collagen (mg/ml)	1.5	1	0.5
10 × MEM	20	20	20	10 × DMEM	20	20	20	10 × EC-Medium	20	20	20
-	-	-	-	NaOH 1M	6	6	5	-	-	-	-
H ₂ O	20	73	127	H ₂ O	14	67	122	H ₂ O	20	73	127
NaHCO ₃ 7.5 %	10	7	3	NaHCO ₃ 7.5 %	10	7	3	NaHCO ₃ 7.5 %	10	7	3
1 × RPMI 1640	50	50	50	1 × DMEM	50	50	50	1 × EC-Medium	50	50	50
Collagen I 3 mg/ml	150	100	50	Collagen I 3 mg/ml	150	100	50	Collagen I 3 mg/ml	150	100	50
Cell suspension	50	50	50	Cell suspension	50	50	50	Cell suspension	50	50	50
Σ	300	300	300	Σ	300	300	300	Σ	300	300	300

Volumes in µl