

Fabrication of collagen I gels

1. General Information

This Application Note describes a simple protocol how to fabricate collagen I gels with different cell culture media. Depending on medium acidity the pH of the mixture has to be adjusted with sodium hydroxide (NaOH). The resulting gel has a concentration of 1.5 mg/mL collagen I. The protocol is elaborated specifically for the given solutions. Application of e.g. a collagen gel of a different manufacturer may influence the whole composition.

2. Material:

- Collagen I, bovine, 3mg/mL (nutacon, 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 1M
- NaHCO₃ 7.5 % (Sigma, S8761)
- L-glutamine 100 × (c.c.pro, Z-10-M)
- Sterile water for molecular biology

3. Fabrication Protocol

- Ingredients are indicated in the order of pipetting.
- Place all solutions at room temperature half an hour before starting the experiment.
- All 10 × media are without supplements; 10 × DMEM needs to be complemented with L-glutamine before usage.
- For addition of supplements (growth factors, inhibitors, serum etc.) add the supplements to the 1 × medium (50 µL) without cells.
Example: Prepare a 10x concentration of the supplement in 30 µL of 1x PBS or 1x medium and add 20 µL of 1x medium.
- After assembling all components fill the gel into the culture vessel within 5 minutes.
- For gelation place the gel in a cell culture incubator (37°C, 5% CO₂) for 45 minutes. In the first minutes the cells are still moving downwards. To avoid a settling of cells on the bottom it may be good to incline the chamber vertically (only for chambers and channels).

4. Pipetting Scheme (1.5 mg/mL collagen I, bovine)

RPMI/MEM	
	µL
10 × MEM	20
-	-
H ₂ O	20
NaHCO ₃ 7.5 %	10
1 × RPMI 1640	50
Collagen I, 3 mg/mL	150
Cell suspension	50
Σ	300

DMEM	
	µL
10 × DMEM	20
NaOH 1M	6
H ₂ O	14
NaHCO ₃ 7.5 %	10
1 × DMEM	50
Collagen I, 3 mg/mL	150
Cell suspension	50
Σ	300

Endothelial Cell Medium	
	µL
10 × EC-Medium	20
-	-
H ₂ O	20
NaHCO ₃ 7.5 %	10
1 × EC-Medium	50
Collagen I, 3 mg/mL	150
Cell suspension	50
Σ	300