

Fabrication of Collagen I Gels

General Information

This Application Note contains protocols for fabricating collagen I gels with varied cell culture media and different types of collagen. There are some general differences between the bovine and rat tail collagens used for gel matrices:

Bovine	Rat Tail
Slow gelation	Fast gelation
Cells will sink down during gelation	Cells will be caught in 3D during gelation
Working at room temperature	Working on ice necessary
Working time (20°C) = max. 5 min	Working time (on ice) = max. 5 min
Pepsinized → highest purity → regular fibrils → less <i>in vivo</i> -like	Non-Pepsinized → high purity → irregular fibrils → more <i>in vivo</i> -like

Important general protocol information:

- Normally, 10 × media are delivered without additive compounds (e.g., L-glutamine or glucose), so those additives will need to be added separately.
- For the addition of supplements (e.g., growth factors, inhibitors, serums, etc.), add the supplements into the 1 × medium without cells (50 µl volume, when preparing 300 µl of gel in total).
For example: Prepare a 10 × concentration of the supplement in 30 µl of 1 × medium. Add 20 µl of 1 × medium. This mixture results in a volume of 50 µl that can be added to the 250 µl gel mix (300 µl in total).
- If cells are desired to be suspended into the gel, then a final cell concentration ranging from 1 to 10 × 10⁶ cells/ml is recommended. If no cells are used, add 1 × medium instead of cell suspension.
- After gelation, collagen fibrils are visible with a 10 × objective lens and phase contrast microscopy.
- If a larger amount of gel is needed, scale up the protocol. Larger volumes might be easier to handle.

Depending on the medium's original acidity, the pH of the final mixture should be adjusted to pH=7.4 using sodium hydroxide (NaOH) and sodium bicarbonate (NaHCO₃) at 37°C and in a 5% CO₂ atmosphere.

These protocols are designed specifically for the given solutions and conditions. Even small changes in the protocol may influence the final pH, thus affecting the correct collagen gelation process. For example, utilizing a collagen from a different manufacturer may influence the whole composition.

As a first trial, we recommend using a 1.5 mg/ml Collagen I gel.

Protocols for two (rat tail collagen) and three (bovine collagen) different media are given in this Application Note. Choose the medium depending on your cell type. DMEM is a standard medium compatible with many cell lines. MEM/RPMI is recommended e.g. for dendritic cells. M199/EC-Medium is recommended for endothelial cells.

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1. Collagen Type I, bovine

1.1 Material:

- Collagen I, bovine, pepsinized, 3 mg/ml (PureCol®, Advanced BioMatrix, 5005-B)
- Media:
 - 10 × MEM (Sigma, M0275), **or**
 - 10 × DMEM (Sigma, D2429), **or**
 - 10 × M199 (Sigma, M0650)
 - RPMI 1640 (Sigma R8758), **or**
 - DMEM (Sigma, D5796), **or**
 - EC-Medium (Promocell, C-22010)
- NaOH in ultrapure H₂O, 1M
- NaHCO₃ 7.5 % (Sigma, S8761)
- Sterile ultrapure water
- Optional: supplements, like L-glutamine

1.2 Fabrication Protocol

1. Place all solutions at room temperature for half an hour before starting the experiment.
2. Determine the final volume of collagen solution to be used (e.g. 300 µl) and the desired, final collagen concentration (e.g., 1.5 mg/ml) by using the table below.
3. Determine the final cell concentration in the gel. Multiply this concentration with a factor of 6 to calculate the required concentration. For example when using ibidi's µ-Slide Chemotaxis 3D, use 18 × 10⁶ cells/ml to reach a final cell concentration of 3 × 10⁶ cells/ml.
4. Prepare a sterile tube with sufficient volume capacity.
5. Mix the gel:
 - a) Add all of the ingredients, as shown in the table below. The ingredients are listed in the order of pipetting.
 - b) After adding the collagen, thoroughly mix the contents of the tube.
 - c) If desired, add the prepared cell suspension to the mixture. If no cells are used, add 1 × medium.
 - d) Thoroughly mix the contents of the tube.
6. Fill the gel into the culture dishes or slides within 5 minutes.
7. For gelation, place the gel in a cell culture incubator (37°C, 5 % CO₂) for 45 minutes. The cells will continue to settle in the first few minutes. Therefore, to avoid having 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				M199/EC-Medium		
<i>Final Collagen I concentration in gel (mg/ml)</i>	1.5	1	0.5		1.5	1	0.5		1.5	1	0.5
10 × MEM	20	20	20	10 × DMEM	20	20	20	10 × M199	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. All ingredients are listed in the order of pipetting

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2. Collagen Type I, rat tail

2.1 Material:

- Collagen I, rat tail, non-pepsinized, 5 mg/ml (ibidi, 50201)
- Media:
 - 10 × DMEM (Sigma, D2429), **or**
 - 10 × M199 (Sigma, M0650)
 - DMEM (Sigma, D5796), **or**
 - EC-Medium (Promocell, C-22010)
- NaOH in ultrapure H₂O, 1M
- NaHCO₃ 7.5 % (Sigma, S8761)
- Sterile ultrapure water
- Optional: supplements, like L-glutamine

2.2. Fabrication Protocol

1. Place all solutions on ice for 10 minutes before starting the experiment.
2. Determine the final volume of collagen solution to be used (e.g., 300 µl) and the desired, final collagen concentration (e.g., 1.5 mg/ml) by using the table below.
3. Determine the final cell concentration in the gel. Multiply this concentration with a factor of 6 to calculate the required concentration. For example when using ibidi's µ-Slide Chemotaxis^{3D} use 18 × 10⁶ cells/ml to reach a final cell concentration of 3 × 10⁶ cells/ml.
4. Place a sterile tube of sufficient volume capacity on ice.
5. Mix the gel:
 - a) Add all of the ingredients, as shown in the table below. The ingredients are listed in the order of pipetting.
 - b) After adding the collagen, thoroughly mix the contents of the tube and then hold on ice.
 - c) If desired, add cell suspension to the mixture. If no cells are used, add 1 × medium.
 - d) Thoroughly mix the contents of the tube and hold on ice.
6. When stored on ice, the collagen solution can be used for a maximum of 5 minutes before partial gelation occurs.
7. For gelation, place the gel in a cell culture incubator (37°C, 5% CO₂) for 30 minutes.

	DMEM					M199/EC-Medium					
<i>Final Collagen I concentration in gel (mg/ml)</i>	3	2	1.5	1	0.5	<i>Final Collagen I concentration in gel (mg/ml)</i>	3	2	1.5	1	0.5
10 × DMEM	21	20	20	20	20	10 × M199	22	20	20	20	20
NaOH 1M	7	6	5	5	4	-	-	-	-	-	-
H ₂ O	-	49	81	112	141	H ₂ O	-	46	79	112	147
NaHCO ₃ 7.5 %	3	5	4	3	5	NaHCO ₃ 7.5 %	18	14	11	8	3
1 × DMEM	39	50	50	50	50	1 × EC-Medium	30	50	50	50	50
Collagen I, 5 mg/ml	180	120	90	60	30	Collagen I, 5 mg/ml	180	120	90	60	30
Cell suspension	50	50	50	50	50	Cell suspension	50	50	50	50	50
Σ	300	300	300	300	300		300	300	300	300	300

Volumes in µl. All ingredients are listed in the order of pipetting.

3. Troubleshooting

Gel Matrix not Homogeneous, Inconsistent Gel or Fibers with Preferred Direction

In order to create consistent migration data, inhomogeneities in gel matrices should absolutely be avoided. The crucial parameters for generating homogeneous gels are: an efficient mixture at the start of the experiment, the quality of the ingredients, the temperature, and the total working time.

Unlike other aqueous solutions, the collagen mixture does not mix itself by diffusion. Make sure to thoroughly combine the viscous collagen mixture, and later the gel mixture, with the cell suspension. Take your time mixing! In addition, excessive up and down pipetting, in combination with stirring with the pipet tip, is also necessary. Finally, make sure to only use freshly aliquoted and non-expired ingredients.

After some time, the collagen mixture will start to gel. Be sure to fill your culture vessel before the gelation starts. The beginning gelation can lead to preferred fibril directions in small channels. However, this should be avoided by (a) lowering the temperature (by working on ice) to slow down the gelation process, or (b) shortening the working time.

pH-Value is not in Physiological Range

If the pH-Value is not correct, adjust it by using sodium hydroxide (NaOH), sodium bicarbonate (NaHCO₃), HEPES, or other additional buffers.

When establishing a new protocol, always control the pH of the gel, after gelation occurs in your incubator!

No Gelation – No Fibrils Visible

To help find a solution, here are some questions to ask:

- *Are you using a 10x objective lens or higher with phase contrast to see the gel structure?*
- *Do you use only freshly aliquoted and non-expired ingredients?*
- *Is the pH in an optimal range? What is the color of the medium?*

Cells Die in the Gel Matrix

pH and salt concentrations can cause many problems in gel matrices. Please test any gel protocol with your cells in an environment suited for this purpose. We recommend small open wells, such as μ -Slide Angiogenesis or μ -Slide 8 well.

Make sure, that the composition of the cell culture medium in the gel is suitable for your cell line. E.g., the 10 x DMEM used in this protocol contains only a low glucose concentration. If your cell line needs a high glucose level, addition of glucose might be necessary for an optimal cell growth.

Cells Do Not Migrate in Gel

To help find a solution, here are some questions to ask:

- *Are the cells seeded in an optimal growth medium?*
- *Is the pH in an optimal range for the cells?*
- *Can the cells degrade the gel matrix?*
- *Is the concentration of gel too high? Is the gel too stiff?*
- *Are there suitable binding motifs for cell attachment?*
- *How strong is the invasion capacity of the cell line used?*