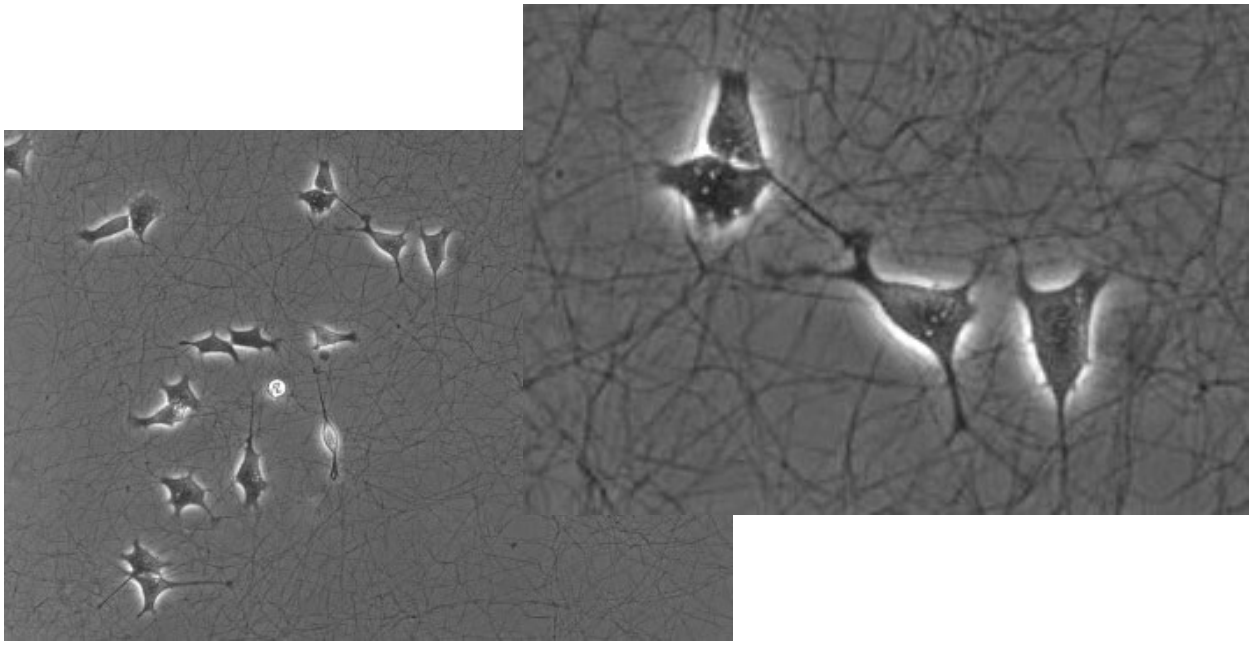
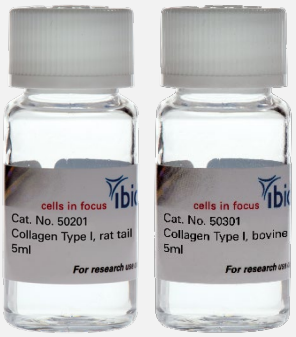
# Preparation of Collagen I Gels

This Application Note contains step-by-step protocols for creating gels from either ibidi Collagen Type I, Rat Tail, or ibidi Collagen Type I, Bovine, using Dulbecco's Modified Eagle Medium (DMEM), Roswell Park Memorial Institute (RPMI 1640) cell culture medium or Phosphate-buffered Saline (PBS). Variations of the protocols below can be calculated using our ibidi Collagen Calculator on ibidi.com.



**ibidi offers various solutions for 3D Collagen gels and Collagen coatings**

- Collagen Type I, Rat Tail
- Collagen Type I, Bovine




**Related Documents**

- Instructions Collagen Type I, Rat Tail, 5 mg/ml (PDF)
- Instructions Collagen Type I, Bovine, 5 mg/ml (PDF)

**Related Software**

- ibidi Collagen Calculator

## Important Information

- The 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 collagen from a different manufacturer may lead to incorrect pH and gelation behavior.
- The protocols are optimized for DMEM or RPMI, which you can choose depending on your cell type. Please note that the 10x media used in this protocol does not contain any $NaHCO_3$; and needs to be added to the gel as described in the protocols below. If a different culture medium is needed, please follow the general gelation protocols in the Instructions of the respective collagen.
- For PBS applications: PBS gels are suitable only for 2D applications with cells grown on the gel surface. PBS gels allow for the highest possible collagen concentration. When using PBS gels, no cell suspension, 1x medium, or $NaHCO_3$ are needed.
- The protocols below use a total collagen gel volume of 300 µl as an example. If a larger amount of gel is needed, you can scale up the protocol. We do not recommend scaling down the volume to less than 300 µl due to an increased impact of pipetting errors.
- Use the ibidi Collagen Calculator for variations of the protocols below, i.e., if different concentrations are needed of the collagen stock solution, the NaOH or the collagen gel.
- All collagen solutions come with a lot-specific concentration, which slightly differs from the nominal concentration. The lot-specific concentration for each batch can be found in the Certificate of Analysis here: Download CoA.
    - If you want the exact same gel protocol independent from the stock solution, use the protocols below with the pre-dilution to 4.0 mg/ml. This pre-dilution ensures that the very same protocol can be used every time.
    - If you want to use the collagen as delivered, use the ibidi Collagen Calculator and download a lot-specific gel protocol. Make sure to update your protocol for each batch using the lot-specific collagen concentration.

## Preparations Before the Experiment

- Define the final Collagen Type I concentration in the gel (see Tables below) depending on your cell type and experiment. We recommend to start with 1.5 mg/ml.
- Check the culture medium supplements that are generally required for growing your cells (e.g., L-glutamine, growth factors, inhibitors, serum, etc.). They should be added in advance to the 1x medium without cells (see Tables below, ingredient #5). The required concentrations should be extrapolated to the total gel volume.

  ***Example:*** *The experiment requires that the final concentration of a supplement in the gel (300 µl total volume) is 1x. To achieve that, prepare 30 µl of 1x medium with 10x concentrated supplement. Then fill up to 50 µl using 20 µl 1x medium. Add these 50 µl (ingredient #5) to the gel mix to get a final gel volume of 300 µl with 1x supplement.*

- If cells should be suspended directly in the collagen gel, define the final cell concentration in the gel (we recommend 1–10 x $10^6$ cells/ml). Multiply the final concentration by 6 to calculate the required cell number to be added.

  ***Example:*** *The experiment requires a final concentration of 1 x $10^6$ cells/ml in the gel (300 µl total volume). To achieve that, prepare 50 µl cell suspension in 1x medium with a concentration of 6 x $10^6$ cells/ml (ingredient #7) and add it to the mix. If no cells are required to be suspended in the gel, add 50 µl 1x medium only instead.*

# 1 Preparing a Gel With Collagen I, Bovine

## 1.1 Material

- Collagen Type I, Bovine, non-pepsinized, 5 mg/ml (ibidi, 50301), **diluted to 4 mg/ml in 0.1 M acetic acid**
- 10x DMEM (Sigma, D2429) **or** 10x RPMI 1640 (Sigma, R1145)
- 1x DMEM (Sigma, D5796) **or** 1x RPMI 1640 (Sigma, R8758)
- Media supplements (e.g., L-glutamine, depending on your cell type)
- NaOH in ultrapure $H_2O$, 1 M
- $NaHCO_3$ 7.5 % (Sigma, S8761)
- Sterile, ultrapure water
- Standard cell culture equipment (sterile working bench, cell detachment kit, culture flasks, pipets, tips, etc.)
- Cell culture vessel to be filled with the gel

## 1.2 Protocol

Perform all the following protocol steps under sterile working conditions.

1. If needed, prepare the cell suspension to be added to the gel (see page 1) and put it under the flow hood at room temperature.
2. If required, add supplements to the 1x cell culture medium (see page 1) and put it on ice in the flow hood.
3. Make sure the Collagen I, Bovine is diluted to 4.0 mg/ml in **0.1 M acetic acid**. Check the Certificate of Analysis (CoA) for the lot-specific collagen concentration. Before diluting, the collagen needs to be mixed actively by pipetting up and down several times. The mixing ensures that a homogeneous solution is created.
4. Place all further ingredients and a sterile tube, with sufficient capacity for the total gel volume, on ice in the flow hood. Unpack the required cell culture vessel and place it in the flow hood as well.
5. Pipet all ingredients except the collagen and the cell suspension in the order listed in Table 1 (use the column for your chosen collagen concentration and cell culture medium) to the tube, keeping it on ice. Mix by pipetting up and down and put back on ice.
6. Add the collagen to the mixture. Mix well by pipetting, keeping the tube on ice.
7. If desired, add the prepared cell suspension to the mixture. If no cells are used, add 1x DMEM or 1x RPMI. In this final step, mixing thoroughly by pipetting up and down several times is crucial.
8. The mixture is now ready to be pipetted into the cell culture vessel of choice for up to 5 minutes. Keep on ice during pipetting.

   **Important Note:** Even on ice, the mixture containing collagen can be used for a maximum of 5 minutes before partial gelation occurs.

9. For gelation, put the cell culture vessel with the gel into a cell culture incubator (37°C, 5% $CO_2$) for 30 minutes.
10. After gelation, collagen fibrils will be visible using phase contrast microscopy with a 10x objective lens.

| | | DMEM | | | | RPMI | | | | PBS | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Final Collagen I, Bovine concentration in gel (mg/ml)* | | | | *Final Collagen I, Bovine concentration in gel (mg/ml)* | | | | *Final Collagen I, Bovine concentration in gel (mg/ml)* | | | |
| | | **2** | **1.5** | **1** | **0.5** | **2** | **1.5** | **1** | **0.5** | **2** | **1.5** | **1** | **0.5** |
| #1 | 10x Medium | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 30.0 | 30.0 | 30.0 | 30.0 |
| #2 | NaOH 1 M | 15.0 | 11.3 | 7.5 | 3.8 | 15.0 | 11.3 | 7.5 | 3.8 | 15.0 | 11.3 | 7.5 | 3.8 |
| #3 | $H_2O$ | 5.1 | 46.3 | 87.6 | 128.8 | 9.7 | 50.9 | 92.2 | 133.4 | 105.0 | 146.2 | 187.5 | 228.7 |
| #4 | $NaHCO_3$ 7.5 % | 9.9 | 9.9 | 9.9 | 9.9 | 5.3 | 5.3 | 5.3 | 5.3 | - | - | - | - |
| #5 | 1x Medium (optionally with supplements) | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | - | - | - | - |
| #6 | Collagen I, Bovine, 4 mg/ml | 150.0 | 112.5 | 75.0 | 37.5 | 150.0 | 112.5 | 75.0 | 37.5 | 150.0 | 112.5 | 75.0 | 37.5 |
| #7 | Cell suspension in 1x Medium | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | - | - | - | - |
| Total volume | | 300 | | | | 300 | | | | 300 | | | |

*Table 1: Pipetting scheme for making gels using Collagen I, **Bovine** with either DMEM, RPMI 1640 or PBS. Volumes in µl. All ingredients are listed in the order of pipetting.*

# 2 Preparing a Gel With Collagen I, Rat Tail

## 2.1 Material

- Collagen Type I, Rat Tail, non-pepsinized, 5 mg/ml (ibidi, 50201), **diluted to 4 mg/ml in 17.5 mM acetic acid**
- 10x DMEM (Sigma, D2429) **or** 10x RPMI 1640 (Sigma, R1145)
- 1x DMEM (Sigma, D5796) **or** 1x RPMI 1640 (Sigma, R8758)
- Media supplements (e.g., L-glutamine, depending on your cell type)
- NaOH in ultrapure $H_2O$, 1 M
- $NaHCO_3$ 7.5 % (Sigma, S8761)
- Sterile, ultrapure water
- Standard cell culture equipment (sterile working bench, cell detachment kit, culture flasks, pipets, tips, etc.)
- Cell culture vessel to be filled with the gel

## 2.2 Protocol

Perform all the following protocol steps under sterile working conditions.

1. If needed, prepare the cell suspension to be added to the gel (see page 1) and put it under the flow hood at room temperature.
2. If required, add supplements to the 1x cell culture medium (see page 1) and put it on ice in the flow hood.
3. Make sure the Collagen I, Rat Tail is diluted to 4.0 mg/ml in **17.5 mM acetic acid**. Check the Certificate of Analysis (CoA) for the lot-specific collagen concentration. Before diluting, the collagen needs to be mixed actively by pipetting up and down several times. The mixing ensures that a homogeneous solution is created.
4. Place all further ingredients and a sterile tube, with sufficient capacity for the total gel volume, on ice in the flow hood. Unpack the required cell culture vessel and place it in the flow hood as well.
5. Pipet all ingredients except the collagen and the cell suspension in the order listed in Table 2 (use the column for your chosen collagen concentration and cell culture medium) to the tube, keeping it on ice. Mix by pipetting up and down and put back on ice.
6. Add the collagen to the mixture. Mix well by pipetting, keeping the tube on ice.
7. If desired, add the prepared cell suspension to the mixture. If no cells are used, add 1x DMEM or 1x RPMI. In this final step, mixing thoroughly by pipetting up and down several times is crucial.
8. The mixture is now ready to be pipetted into the cell culture vessel of choice for 5 minutes. Keep on ice during pipetting.

   **Important Note:** Even on ice, the mixture containing collagen can be used for a maximum of 5 minutes before partial gelation occurs.

9. For gelation, put the cell culture vessel with the gel into a cell culture incubator (37°C, 5% $CO_2$) for 30 minutes.
10. After gelation, collagen fibrils will be visible using phase contrast microscopy with a 10x objective lens.

| | | DMEM | | | | RPMI | | | | PBS | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Final Collagen I, Rat Tail concentration in gel (mg/ml)* | | | | *Final Collagen I, Rat Tail concentration in gel (mg/ml)* | | | | *Final Collagen I, Rat Tail concentration in gel (mg/ml)* | | | |
| | | **2** | **1.5** | **1** | **0.5** | **2** | **1.5** | **1** | **0.5** | **2** | **1.5** | **1** | **0.5** |
| #1 | 10x Medium | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 30.0 | 30.0 | 30.0 | 30.0 |
| #2 | NaOH 1 M | 2.6 | 2.0 | 1.3 | 0.7 | 2.6 | 2.0 | 1.3 | 0.7 | 2.6 | 2.0 | 1.3 | 0.7 |
| #3 | $H_2O$ | 17.5 | 55.6 | 93.8 | 131.9 | 22.1 | 60.2 | 98.4 | 136.5 | 117.4 | 155.5 | 193.7 | 231.8 |
| #4 | $NaHCO_3$ 7.5 % | 9.9 | 9.9 | 9.9 | 9.9 | 5.3 | 5.3 | 5.3 | 5.3 | - | - | - | - |
| #5 | 1x Medium (optionally with supplements) | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | - | - | - | - |
| #6 | Collagen I, Rat Tail, 4 mg/ml | 150.0 | 112.5 | 75.0 | 37.5 | 150.0 | 112.5 | 75.0 | 37.5 | 150.0 | 112.5 | 75.0 | 37.5 |
| #7 | Cell suspension in 1x Medium | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | - | - | - | - |
| Total volume | | 300 | | | | 300 | | | | 300 | | | |

*Table 2: Pipetting scheme for making gels using Collagen I, **Rat Tail** with either DMEM, RPMI 1640 or PBS. Volumes in µl. All ingredients are listed in the order of pipetting.*

# 3 Troubleshooting

## Non-Homogeneous Gel Matrix, Incomplete Gelation, or Heterogeneous Fiber Orientations with Preferred Direction

For consistent results, inhomogeneities in gel matrices should be absolutely avoided. This chapter lists crucial parameters for generating homogeneous gels.

**Homogeneous collagen and gel solution:** Collagen is a viscous material that must be mixed actively by pipetting. Mix the collagen thoroughly before the experiment by pipetting up and down several times. During gel preparation, excessive up and down pipetting, plus stirring with the pipet tip, are necessary, especially after adding the collagen and the cell suspension (step 7 in the protocol). This ensures that a homogeneous solution is created. Vortexing is not recommended for mixing viscous liquids.

**High quality ingredients:** Ensure all ingredients are freshly aliquoted and not expired.

**Working temperature and time:** During gel preparation, perform all pipetting steps on ice with pre-cooled ingredients to slow down the gelation process. Work quickly, especially after the collagen has been added to the gel mixture, since after some time, the collagen mixture will start to gel. Be sure to fill your culture vessel before the gelation starts.

### No Gelation Occurs and no Visible Fibrils

Macroscopically, the collagen gelation is barely visible. This is because collagen gels are very, very soft. Unlike other gels (e.g., agarose gels for electrophoresis), they do not give any resistance to a pipet tip touching the gel. The collagen fibrils only create slight turbidity, which is visible to the naked eye. Further, after gelation, the solution is not a flowing liquid anymore.

If you are in doubt about the gelation of your gel, use a phase contrast microscope to see the fibrils with a 10x objective lens. Please note that the fibrils are barely visible in brightfield mode.

Make sure that all ingredients are freshly prepared and not expired.

Please also make sure that your pipet is working properly. Absolute precision is necessary for pipetting the low volumes used in this protocol.

### Dying Cells in the Gel Matrix

Incorrect pH and salt concentrations can cause problems in gel matrices. Please test any gel protocol with your cells in a simple cell culture vessel. We recommend small open wells, such as the µ-Slide 15 Well 3D (ibidi, 81506) or the µ-Slide 8 Well high (ibidi, 80806).

Make sure that the composition of the cell culture medium in the gel is suitable for your cell line.

> ***Example:*** *The 10x DMEM used in this protocol contains a low glucose concentration. If your cell line needs a high glucose level, the addition of glucose might be necessary for optimal cell growth.*

When establishing a new protocol, always control the pH of the gel after gelation using pH paper. If the pH is not correct, adjust it by changing the sodium hydroxide (NaOH) concentration.

### Cells Do Not Migrate in the Gel

In some applications, cells should migrate through the gel matrix in a 3-dimensional manner. Please consider the following topics if problems occur:

- Are the cells seeded in an optimal growth medium?
- Is the pH in an optimal range for the cells?
- Is the gel concentration too high? Is the gel too stiff?
- Are there suitable binding motifs for cell attachment? Is collagen a suited matrix, or are other ECM compositions necessary?
- How strong is the invasion capacity of the cell line used?