Instructions

Collagen Type I, bovine, 5 mg/ml

Type I Collagen is a major structural component of the extracellular matrix (ECM). Therefore, this fibrous protein is often used in three-dimensional (3D) collagen gels that simulate the *in vivo* cell environment better than the traditional 2D systems. Additionally, Collagen I is ideal for coating surfaces, as it can form thin layers for culturing cells.
ibidi's Type I Collagen is a state-of-the-art, purified protein extracted from bovine hide without pepsinization. It is intended to be used in cell-based assays, like 3D gels or coating of lab culture-ware.

Please read the following Application Note for specific gel protocols using DMEM and RPMI 1640:
Application Note 26: "Preparation of Collagen I Gels"

Overview

This document is applicable to the following product numbers:

Cat. No.	Product Name
50301	**Collagen Type I, bovine, 5 mg/ml, 1 × 5 ml**: non–pepsinized
50302	**Collagen Type I, bovine, 5 mg/ml, 4 × 5 ml**: non–pepsinized
50303	**Collagen Type I, bovine, 5 mg/ml, 1 × 100 ml**: non–pepsinized

Specifications

Collagen Type I, bovine	
Source	Bovine dermis, age of calves: $\leq$ 6 months
Appearance	Optically clear viscous liquid
Extraction	Acid, non-pepsinized
Sterility	Sterile, for cell culture
Contaminants	Negative for bacterial and fungal contamination
Growth factors	None
Formulation	Supplied in 0.1 M acetic acid
pH	3.3–3.7

Material

Collagen is a fibrous protein that consists of three α-chains. They combine to create a rope-like triple helix, thus providing tensile strength to the extracellular matrix (ECM). The triple helices aggregate and form fibrils in a self-organized manner. *In vivo*, the fibrils aggregate into fibers to form tissue such as tendon or dermis.

Unlike pepsin-extracted collagen, the ibidi Collagen Type I is acid-extracted. This, and the very mild manufacturing process, preserve a maximal nativity.

Shipping and Storage

Shipping conditions	Ambient temperature *
Storage conditions	2°C – 8°C
Shelf life	Under proper storage conditions as indicated on vial

*Shipped with additional ice pack to assure temperature below 25°C.

Applications

At a neutral pH, Type I Collagen will form a 3D gel, similar to the animal extracellular matrix. 3D gels allow to study the effects of the mechanical properties of the ECM on cell development, as well as chemotaxis, migration, and morphology. Unlike 2D systems, 3D environments allow cell extensions to simultaneously utilize integrins all over the cell membrane. This results in the activation of specific signaling pathways. Gel stiffness, or rigidity, affects cell migration differently in 3D than in 2D environments. Furthermore, integrin-independent mechanical interactions, resulting from the entanglement of matrix fibrils with cell extensions, are possible in 3D systems, but not in 2D systems where the cells are attached to a flat surface.

Additionally, Collagen I is ideal for the thin coating of surfaces in 2D environments. It promotes cell adhesion for numerous cell types in a 2D culture.

Both the 2D and 3D applications include the study of tumor cell invasion, migration, and the chemotaxis of macrophages and/or monocytes.

Notes:

The viscosity of the undiluted collagen solution is high. Handle the undiluted collagen with pipets for high viscosity solutions only. Among others, we recommend Eppendorf Visco Tips or Gilson Microman E.

Being a product of natural origin, the rate of gel formation, consistency, and clarity may vary between lots.

The collagen gelation is pH-driven. Temperature cannot cause gelation, but high temperatures (>37°C) and irradiation can cause protein denaturation.

The temperature during gelation influences several properties of the 3D gel. Agitation of the gel during formation, exposure to ultraviolet light, and temperature extremes can influence the integrity of the gel.

Aliquoting

The viscosity of the undiluted collagen solution is high. Handle the undiluted collagen with pipets for high viscosity solutions only. Among others, we recommend Eppendorf Visco Tips or Gilson Microman E.

Upon arrival, store the collagen solution at 4°C and aliquote within 48 hours.

1. Remove the solution from the vial and transfer to a centrifuge tube. Use a pipet for high viscosity solutions. Do not mind air bubbles at this point.

2. Mix well by pipetting the solution up and down. This ensures creating a homogeneous solution by shear-thinning. Do not vortex. Vortexing does not mix viscous solutions well. Do not mind air bubbles at this point.

3. Spin down the centrifuge tube at 200 × g for 2 min to remove all air bubbles.

4. Aliquot into one time use aliquotes using appropriate volumes. Optionally, dilute in 0.1 M acetic acid before aliquoting. Mix well.

5. Store the aliquotes at 2°C – 8°C.

Notes:

Do not freeze the collagen solution.

Collagen is insoluble at a neutral pH. It can be diluted in 0.1 M acetic acid.

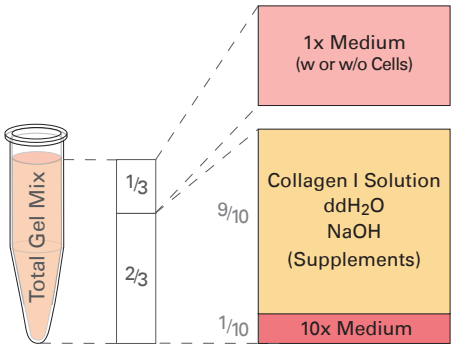
Specific 3D Gel Protocols for DMEM and RPMI 1640

Step-by-step protocols for creating gels using either Dulbecco's Modified Eagle Medium (DMEM) or Roswell Park Memorial Institute (RPMI 1640) cell culture medium can be found in:

Application Note 26: "Preparation of Collagen I Gels"

General 3D Gel Protocol

Use these recommendations as guidelines to elaborate the optimal gelation protocol for your culture system.



Notes:

The salt concentration in the final gel mix needs to be 1×.

The final pH needs to be 7.2–7.4.

If supplements (e.g., growth factors, inhibitors, sera, L–glutamine, etc.) are added, make sure the final concentation is 1×.

Always add the cell suspension after pH adjustment.

1. Place the following on ice:
   - Sterile ddH_2O
   - Sterile 1M NaOH

- 10× medium (or 10× buffer)
- 1× medium (or 1× buffer)
- Additional buffers (e.g. $NaHCO_3$)
- Collagen solution (diluted to 4.0 mg/ml)

2. Calculate the volume of collagen solution to be used:

$$V_{Collagen}\,[ml] = \frac{V_{Gel,\,final}\,[ml] \cdot C_{Collagen,\,final}\,[mg/ml]}{C_{Collagen,\,solution}\,[mg/ml]}$$

3. Calculate the volume of the 10× medium to be used:

$$V_{10\times Medium}\,[ml] = \frac{2}{3} \cdot V_{Gel,\,final}\,[ml] \cdot \frac{1}{10}$$

4. Place a sterile tube, of sufficient capacity, on ice.
5. Perform these steps, in the following order, under sterile conditions:
   Note: On ice, the mixture containing the collagen can be used for a maximum of 5 minutes before partial gelation occurs.
   (a) Pipet the 10× medium into the tube.
   (b) Add sterile, ice-cold 1M NaOH to the 10× medium to adjust the pH to an alkaline milieu. The exact volume is determined by measuring the pH of the final gel mixture (after step 5g).
   (c) Optionally, add additional buffers (e.g. $NaHCO_3$) if not contained in the 10× medium.
   (d) Add ddH_2O to match the 2/3 of the final gel volume.
   (e) Supplement the mixture with 1× medium (1/3 of the final volume). If addition of cells is desired, only add half of this volume here. Add the cell suspension after step 5g.
   (f) Dilute the collagen to 4.0 mg/ml in 0.1 M acetic acid. Check the Certificate of Analysis (CoA) for the lot-specific collagen concentration. Mix well.
   (g) Add Collagen I to the tube. Mix the contents of the tube thoroughly and place on ice.
   (h) If desired, add a cell suspension to the mixture.
   (i) Mix the contents of the tube thoroughly and place on ice.
   (j) The mixture is now ready to be pipetted into the cell culture vessel of choice for 5 minutes. Keep on ice during pipetting.
   (k) For gelation, place the mixture in a cell culture incubator (37°C, 5% CO_2) for 30 minutes.

Notes:

For the addition of supplements (e.g., growth factors, inhibitors, sera, L–glutamine, etc.), add the supplements to the 1× medium without cells.

Keep in mind that the concentration of the cell suspension is diluted, when added to the mixture.

Final cell concentrations ranging from 1 to 20 × 10^6 cells/ml are recommended.

A collagen gel is no longer pipettable because the structure will be damaged by the pipet tip and the suction force.

Thin Coating Procedure

We recommend using Collagen I as a thin coating at 5 µg/cm^2. Please use this only as a guideline value. Optimization of the desired protein concentration might be required. Also, further dilution may be desired, depending on the cell system.
For a complete coating protocol for ibidi products see our Application Note 08: "Coating Protocols for ibidi Labware".

Preparing Acetic Acid Solution

The collagen is dissolved in 0.1 M acetic acid. To prepare this solution, use the following procedure:

1. Use 17.5 M stock solution of acetic acid (e.g. A6283, Sigma–Aldrich).
2. Prepare sterile, double distilled water (ddH_2O) for cell culture.
3. Dilute the acetic acid stock solution 1:175 in ddH_2O.

Coating Protocol

1. Determine the volume of the dish or channel to be coated.
2. Determine the coating area $A_{coating}$ (i.e., the complete area that comes in contact with fluids).
3. Calculate the required collagen concentration:

$$C_{Collagen}[\mu g/ml] = \frac{A_{coating}[cm^2] \cdot 5\ \mu g/cm^2}{V[ml]}$$

4. Dilute collagen to the calculated concentration, using 0.1 M acetic acid. Collagen is insoluble at neutral pH.
5. Fill the dish or channel.
6. Incubate at room temperature for one hour.
7. Fully aspirate the channel or well volume.
8. Carefully rinse with PBS or serum-free medium.
9. Wells or channels are now ready for use. Optionally, air-dry them at room temperature.
10. Store under sterile conditions and use as soon as possible.

Examples

The table below shows some examples, which concentration of Collagen I is necessary to coat the surface with 5 µg/cm^2. In case of the multi-well plates, please use the following concentration as guideline values only.

Please keep in mind that all cell culture devices are coated on the entire surface that is wetted by the liquid (coating area). That includes the growth area, the side walls and, in case of channels, the channel's ceiling.

	Growth area per well [cm^2]	Coating area per well [cm^2]	Volume [ml]	Collagen Concentration [µg/ml]
ibidi µ-Slide 8 Well	1.1	2.2	0.3	35
ibidi µ-Slide VI $^{0.4}$	0.6	1.2	0.03	200
6 well plate	10.0	12.0	4.0	15
12 well plate	3.5	5.0	1.5	17
24 well plate	1.9	4.3	1.0	20
48 well plate	1.0	4.0	0.8	25
96 well plate	0.55	2.4	0.3	35
384 well plate	0.11	0.8	0.05	80
Culture Flask 75 cm^2	75.0	85.0	10.0	42
Culture Flask 25 cm^2	25.0	30.0	4.0	38

Ordering Information

ibidi provides Collagen Type I from rat tail and from bovine origin.

Cat. No.	Description
50201	**Collagen Type I, rat tail, 5 mg/ml, 1 × 5 ml**: non–pepsinized
50202	**Collagen Type I, rat tail, 5 mg/ml, 4 × 5 ml**: non–pepsinized
50203	**Collagen Type I, rat tail, 5 mg/ml, 1 × 100 ml**: non–pepsinized
50204	**Collagen Type I, rat tail, 10 mg/ml, 1 × 5 ml**: non–pepsinized
50205	**Collagen Type I, rat tail, 10 mg/ml, 4 × 5 ml**: non–pepsinized
50206	**Collagen Type I, rat tail, 10 mg/ml, 1 × 100 ml**: non–pepsinized
50301	**Collagen Type I, bovine, 5 mg/ml, 1 × 5 ml**: non–pepsinized
50302	**Collagen Type I, bovine, 5 mg/ml, 4 × 5 ml**: non–pepsinized
50303	**Collagen Type I, bovine, 5 mg/ml, 1 × 100 ml**: non–pepsinized

For research use only!

Further information can be found at ibidi.com. For questions and suggestions please contact us by e-mail *info@ibidi.de* or by telephone +49 (0)89/520 4617 0.