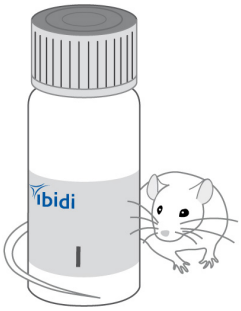
Instructions

Collagen Type I, rat tail, 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 rat tail tendons without pepsinization. It is specially engineered for ibidi's cell–based assays (e.g., chemotaxis assays in 3D gels, or 2D coating of lab cultureware).

Material

Collagen is a fibrous protein that consists of three α–chains, which can be combined to form a rope–like triple helix, thus providing tensile strength to the extracelluar matrix (ECM). The α–chains contain GXY repeats: glycine (G) is an amino acid that fits well into the triple helix. Typically, X and Y represent hydroxyproline and proline, which are key components for the stability of collagen.

Type I is the most common fibrillar collagen, and is mostly found in skin, bone, tendons, and other connective tissues. Due to the acid extraction and protein purification, this collagen is considered to be free of any growth factors.

Specifications

Collagen Type I, rat tail	
Source	Rat tail tendon
Appearance	Optically clear viscous liquid
Extraction	Acid, non–pepsinized
Purity	$>$ 90 % by SDS PAGE
Sterility	Sterile, for cell culture
Contaminants	Negative for DNA, bacteria, fungi, and mycoplasma
Growth factors	None
Formulation	Supplied in 17.5 mM acetic acid ($\sim$ 0.1%)
pH	pH $\sim$ 3.8
Functional control	3D gelling and 2D coating test in cell culture
Degeneration	Collagenase NB 4 (Standard Grade) from *C. histolyticum* (Serva, Cat–No. 17454.02)
Antibody staining	Antibody for rat skin, e.g. BIOLOGO, Cat–No. CO20141-0.1

Applications

At a neutral pH and 37°C, Type I Collagen will form a 3D gel, similar to the animal extracellular matrix. 3D gels allow you 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 on both the dorsal and ventral cell surfaces. 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.

Shipping and Storage

Shipping conditions	Dry ice
Storage conditions	-20°C
Shelf life	Under proper storage conditions as indicated on vial

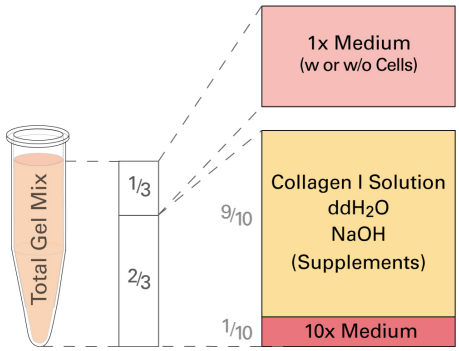
Notes:

Thaw the product on ice and aliquot to appropriate volumes. Always refrigerate stock or diluted solutions. Do not thaw and refreeze multiple times.

Collagen denatures when exposed to high temperatures or irradiation. Also, the collagen is insoluble at a neutral pH. It can be diluted in 17.5 mM acetic acid.

General 3D Gel Protocol

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



Notes:

The final medium concentration in the resulting gel needs to be 1×.

The final pH needs to be 7.2–7.4.

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 (5 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) Add Collagen I to the tube.
   (g) Mix the contents of the tube thoroughly and place on ice.
   (h) If desired, add cell suspension to mixture.
   (i) Mix the contents of the tube thoroughly and place on ice.
6. For gelation, place the gel in a cell culture incubator (37°C, 5% CO_2) for 30 minutes.

Notes:

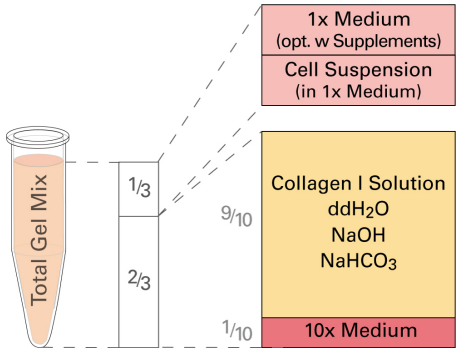
For the addition of supplements (e.g., growth factors, inhibitors, serums, L–glutamine etc.), add the supplements to the 1× medium without cells.

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.

Specific 3D Gel Protocol for DMEM

For cells which are cultured in DMEM the following gel protocol is recommended.



1. Place the following on ice:
   - Sterile ddH_2O
   - Sterile 1M NaOH
   - 10× DMEM
   - DMEM (with 10% FCS)
   - Sterile $NaHCO_3$ 7.5%
   - Collagen solution (5 mg/ml)
2. Determine the final volume of collagen solution to be used and the desired, final collagen concentration using the table below.
3. Determine the final cell concentration in the gel. Multiply this concentration with a factor 6 to calculate the required concentration. For ibidi's µ–Slide Chemotaxis use 18×10^6 cells/ml to reach a final cell concentration of 3×10^6 cells/ml.
4. Place a sterile tube of sufficient volume capacity on ice.
5. Determine the volume of collagen to be used using the table below.
6. Perform the following steps 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) 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, mix the contents of the tube and hold on ice.
   (c) If desired, add cell suspension to mixture. If no cells are used, add 1× DMEM.
   (d) Mix the contents of the tube and hold on ice.
7. For gelation, place the gel in a cell culture incubator (37°C, 5% CO_2) for 30 minutes.

Collagen, final [mg/ml]	0.5	1	1.5	2	3
	Volumes in µl				
10× DMEM	20	20	20	20	21
1M NaOH	4	5	5	6	7
ddH_2O	141	112	81	49	-
$NaHCO_3$ 7,5%	5	3	4	5	3
1× DMEM	50	50	50	50	39
Collagen I (5 mg/ml)	30	60	90	120	180
Cell suspension	50	50	50	50	50
Total	300	300	300	300	300

Notes:

For the addition of supplements (e.g., growth factors, inhibitors, serums, L–glutamine etc.), add the supplements to the 1× medium (50 µl) without cells.

Keep in mind that the used cell concentration of the cell suspension is diluted by a factor of 6.

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

If a larger amount of gel is needed, scale up the protocol. Larger volumes might be easier to handle.

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. An optimization for the desired protein concentration might be required. Also, further dilution may be desired, depending on the cell system.

Preparing Acetic Acid Solution

The rat tail collagen is dissolved in 17.5 mM acetic acid (∼ 0.1%). 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 1:1000 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 17.5 mM 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.

For a complete coating protocol for ibidi products see Application Note 08 on www.ibidi.com.

	Growth area per well [cm^2]	Coating area per well [cm^2]	Volume [ml]	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	250
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	70
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

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

For research use only!

Further technical specifications can be found at www.ibidi.com. For questions and suggestions please contact us by e-mail *info@ibidi.de* or by telephone +49 (0)89/520 4617 0. All products are developed and produced in Germany.