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).

Please read the following Application Note for more detailed information:
Application Note 08 “Coating protocols for ibidi labware products”

### 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 polymerize into fibers to form tissue such as tendon or dermis.

Unlike pepsin-extracted collagen (usually from bovine skin), the ibidi Collagen Type I, Rat Tail is acid-extracted. This, and the very mild manufacturing process, preserve a maximal nativity.

### Notes:

The ibidi Collagen Type I, rat tail has a very high viscosity due to the high level of native cross-links and the high protein concentration, especially at 10 mg/ml.

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

The viscosity of collagens from different suppliers might be lower due to their lower level of natural cross-linking.

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

The temperature during polymerization influences the properties of the 3D gel.

### Specifications

<table>
<thead>
<tr>
<th>Collagen Type I, rat tail</th>
<th>Source</th>
<th>Rat tail tendon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Optically clear viscous liquid</td>
<td></td>
</tr>
<tr>
<td>Extraction</td>
<td>Acid, non-pepsinized</td>
<td></td>
</tr>
<tr>
<td>Purity</td>
<td>&gt; 90 % by SDS PAGE</td>
<td></td>
</tr>
<tr>
<td>Sterility</td>
<td>Sterile, for cell culture</td>
<td></td>
</tr>
<tr>
<td>Contaminants</td>
<td>Negative for DNA, bacteria, fungi, and mycoplasma</td>
<td></td>
</tr>
<tr>
<td>Growth factors</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Formulation</td>
<td>Supplied in 17.5 mM acetic acid (~0.1%)</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>3.4–4.2</td>
<td></td>
</tr>
<tr>
<td>Degradation</td>
<td>Collagenase NB 4 (Standard Grade) from <em>C. histolyticum</em> (Serva, Cat-No. 17454.02)</td>
<td></td>
</tr>
<tr>
<td>Antibody staining</td>
<td>Antibody for rat skin, e.g. BIOL-OGO, Cat-No. CO20141-0.1</td>
<td></td>
</tr>
</tbody>
</table>

### Shipping and Storage

<table>
<thead>
<tr>
<th>Shipping conditions</th>
<th>Dry ice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage conditions</td>
<td>–20°C</td>
</tr>
<tr>
<td>Shelf life</td>
<td>Under proper storage conditions as indicated on vial</td>
</tr>
</tbody>
</table>

### 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.

Thawing and Aliquoting

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

1. Thaw the product in the refrigerator at 4°C overnight.
2. Make sure the solution is completely thawed. Optionally, spin down at 700 xg for 10 min to remove small air bubbles.
3. Aliquot into one time use aliquotes using appropriate volumes. Optionally, dilute in 17.5 mM acetic acid before aliquoting. Mix well.
4. Refreeze aliquots or diluted solutions immediately.

Notes:
Do not use partially thawed solutions.
Do not thaw and refreeze multiple times.
Collagen is insoluble at a neutral pH. It can be diluted in 17.5 mM acetic acid.

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 (ddH₂O) for cell culture.
3. Dilute the acetic acid stock solution 1:1000 in ddH₂O.

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/cm². 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.
### Universal Polymerization Protocol

If you want to prepare 3D gels either with or without cells, we recommend using our 5 mg/ml Collagen I products. See Ordering Information for details. Different protocols are available in the Collagen Type I, rat tail, 5 mg/ml instructions.

Due to its high concentration (and high viscosity), the 10 mg/ml collagen solution is preferable for special applications, such as scaffolds and bioprinting.

#### Note:

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

#### Important information:

Due to the rigid and long triple helical conformation of the collagen molecule, the viscosity of collagen solution is extremely high at more than 4 mg/ml, and is hard to handle at higher concentrations.
### Ordering Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>50201</td>
<td>Collagen Type I, rat tail, 5 mg/ml, 1 × 5 ml: non–pepsinized</td>
</tr>
<tr>
<td>50202</td>
<td>Collagen Type I, rat tail, 5 mg/ml, 4 × 5 ml: non–pepsinized</td>
</tr>
<tr>
<td>50203</td>
<td>Collagen Type I, rat tail, 5 mg/ml, 1 × 100 ml: non–pepsinized</td>
</tr>
<tr>
<td>50204</td>
<td>Collagen Type I, rat tail, 10 mg/ml, 1 × 5 ml: non–pepsinized</td>
</tr>
<tr>
<td>50205</td>
<td>Collagen Type I, rat tail, 10 mg/ml, 4 × 5 ml: non–pepsinized</td>
</tr>
<tr>
<td>50206</td>
<td>Collagen Type I, rat tail, 10 mg/ml, 1 × 100 ml: non–pepsinized</td>
</tr>
</tbody>
</table>

For research use only!

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

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