Collagen (rat)

From rat tail tendon
Lyophilizate, cell culture grade

Cat. No. 11 179 179 001
3 vials of 10 mg

**Product overview**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Lyophilized, cell culture grade, free of microorganisms as tested using an established microbiological enumeration test.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation</td>
<td>Collagen is purified from rat tail tendon by a modification of the method of Bornstein (1, 2). Collagen from rat tail consists mainly of type I collagen.</td>
</tr>
<tr>
<td>Reconstitution</td>
<td>It is recommended to dissolve the lyophilizate in sterile 0.2% acetic acid (v/v). For best results, dissolve the cell culture grade lyophilizate in sterile 0.2% acetic acid (v/v). In sterile 0.2% acetic acid (v/v), this product can be used in cell culture without further filtration. For the preparation of collagen gels, the content of the bottle should be dissolved in 3.3 ml sterile 0.2% acetic acid (v/v) each. This results in a final concentration of 3 mg/ml. For coating culture dishes the final concentration should be 1 – 2 mg/ml. For dissolving: do not stir, just pour the acetic acid onto the lyophilizate and let it stand for several hours until it has dissolved. For fully dissolve the product an incubation for up to a maximum of 24 h at +15 to +25°C may be required.</td>
</tr>
</tbody>
</table>

**Biological activity**

The collagen is tested for the promotion of adherence of human umbilical vein endothelial cells (HUVEC).

**Working concentration**

The recommended concentration for the coating of cell culture vessels is 5 µg/cm².

For the production of collagen gels a final concentration of 2 – 3 mg/ml is used.

**Species specificity**

Collagen (rat tail) is active on most vertebrate cells.

**Application**

- Collagen (rat tail) is used as a substrate for the culture of cells.
- Collagen (rat tail) can either be used for the coating of surfaces (culture vessels, slides, cover slips, etc.) or for the preparation of collagen gels.

**Storage/ stability**

Stable at +2 to +8°C until the expiration date printed on the label. The reconstituted solution in sterile acetic acid is also stable at +2 to +8°C.

**Procedures and required material**

**Coating cell culture dishes with collagen**

**Overview**

Rat tail collagen is useful for cultivating cells which need a substrate to grow and to proliferate. Collagen as a substrate is used in the form of either a thin film of dried collagen or a hydrated collagen gel (3–12). A thin film of dried collagen is prepared by spreading the collagen solution onto the surface of a dish and air dried.

**Additional reagents required**

- sterile 0.2% acetic acid (v/v)
- medium or buffer for washing purposes

**Procedure**

Coating of cell culture dishes with collagen (= collagen film). Please refer to the following table.

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dissolve each vial of the lyophilized Collagen with 5 ml sterile 0.2% acetic acid (v/v) to give a final concentration of 2 mg/ml.</td>
</tr>
<tr>
<td>2</td>
<td>Pipette 2.5 µl of this solution (2 mg/ml) per 1 cm² surface area to be coated (5 µg/cm²). This can be increased or decreased to fit the application.</td>
</tr>
<tr>
<td>3</td>
<td>Carefully spread the collagen solution with a sterile rubber policeman on the bottom of the culture dish.</td>
</tr>
<tr>
<td>4</td>
<td>Air dry for about 60 min at +15 to +25°C in the laminar flow hood.</td>
</tr>
<tr>
<td>5</td>
<td>It is possible, but not essential, to wash the coated surface with medium or buffer.</td>
</tr>
<tr>
<td>6</td>
<td>The dishes can be used immediately or stored under sterile conditions.</td>
</tr>
</tbody>
</table>

**Preparation of collagen gels**

**Overview**

Collagen gels can be prepared by a number of different procedures.

One method consists of exposing ammonia vapor to the collagen solution (see method 1). Another method consists of adjusting the pH and ionic strength of the collagen solution (see method 2).

For the three dimensional culture of various cell types, the rat tail collagen gel has proved to be an easy and useful system. For the two systems working instructions are given below. Work should be done under a laminar flow hood.

**Handling instructions**

- To allow for the formation of a homogenous gel and to avoid clump formation do not or only very carefully move culture vessels during gel formation.
- After attachment of the cells, the gel can be detached from the dish with a sterile pipette tip and allowed to float in the medium.

**Additional material required**

- 0.2% acetic acid (v/v), pH 3.0 or 1 mM hydrochloric acid, pH 3.0
- phenolred (optional)
- 25% ammonia solution (v/v)
- cell culture medium
- 35-mm petri dish
- 60-mm petri dish
Method 1

Please refer to the following table.

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dissolve each vial of the lyophilized Collagen in 3.3 ml sterile 0.2% acetic acid (v/v), pH 3.0 or 1 mM hydrochloric acid, pH 3.0. This gives a final concentration of 3 mg/ml.</td>
</tr>
<tr>
<td>2</td>
<td>Let it stand overnight for swelling up.</td>
</tr>
<tr>
<td>3</td>
<td>Pipette 100 μl of this collagen solution per 1 cm² surface area to be covered into the culture vessel. This gives an approximately 1 mm thick collagen gel layer.</td>
</tr>
<tr>
<td>4</td>
<td>The collagen solution is then exposed to ammonia vapors at +15 to +25°C or +37°C.</td>
</tr>
<tr>
<td>5</td>
<td>An example for preparing a collagen gel in a 35-mm dish: • Pipette 100 μl 25% ammonia solution (v/v) into a 60-mm petri dish. • A 35-mm dish containing 1 ml collagen solution is placed in the 60-mm dish which is then closed. If phenolred is present in the collagen solution, the change of the pH can be easily observed (color change to neutral). If there is no pH indicator in the collagen solution the 35-mm dish should be removed after max. 2 min. If the gel layer is thicker the gel needs longer to solidify.</td>
</tr>
<tr>
<td>6</td>
<td>After about 2 min the gel should be solidified. Equilibrate the gel with an appropriate amount of medium for 30 min. This is necessary to remove excess ammonia which is toxic to the cells. A longer period for equilibration is needed for a thicker collagen layer.</td>
</tr>
<tr>
<td>7</td>
<td>Aspirate the medium.</td>
</tr>
<tr>
<td>8</td>
<td>Then the cell can be seeded onto the gel.</td>
</tr>
</tbody>
</table>

Additional reagents required
- sterile 0.2% acetic acid (v/v), pH 3.0 or 1 mM hydrochloric acid, pH 3.0.
- sterile 10 × (5 ×) concentrated medium, with sodium bicarbonate, pH 7.4.
- Check the instructions provided by the supplier of the 10 × (5 ×) concentrated medium regarding the appropriate amount of sodium bicarbonate for the specific medium used.
- sterile (10 ×) 0.2 M Hepes, pH 7.3 [dilute 1 M Hepes tissue culture tested, 1 : 5 with sterile double dist. water].

Method 2

Please refer to the following table.

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dissolve each vial of the lyophilized Collagen in 3.3 ml sterile 0.2% acetic acid (v/v), pH 3.0 or 1 mM hydrochloric acid, pH 3.0. This gives a final concentration of 3 mg/ml.</td>
</tr>
<tr>
<td>2</td>
<td>Mix the following solutions at +2 to +8°C, avoid the formation of air bubbles: • 1 (or 2) parts of sterile 10 × (5 ×) concentrated medium, with sodium bicarbonate, pH 7.4. • 1 part of sterile (10 ×) 0.2 M Hepes, pH 7.3 [dilute 1 M Hepes tissue culture tested, 1 : 5 with sterile double dist. water]. • 8 (or 7) parts of the sterile collagen solution. The mixture remains liquid at +2 to +8°C.</td>
</tr>
<tr>
<td>3</td>
<td>Pipette 100 μl of this neutralized collagen solution per 1 cm² surface area to be covered into the culture vessel. This gives an approximately 1 mm thick collagen gel layer.</td>
</tr>
<tr>
<td>4</td>
<td>Incubate for approx. 2–3 h at +15 to +25°C or at 37°C in a humidified atmosphere to allow gel formation. The culture dishes can be stored at +2 to +8°C under sterile conditions.</td>
</tr>
<tr>
<td>5</td>
<td>Before inoculating the cell suspension it is recommended to cover the gel with medium for about 15 min for equilibration.</td>
</tr>
</tbody>
</table>

Subculture of cells
To subculture the cells, the collagen gel can be digested with collagenase (e.g., collagenase A*, 0.1% in HBSS for approximately 10 to 20 min or until the gel is digested at +37°C). The cells can be recovered and washed by centrifugation. Cells must be washed, to be completely free of collagenase if they are to be cultured on collagen again. If the cells are in clumps, single-cell suspensions can be prepared by further digestion with trypsin/EDTA* or dispase*.

Embedding cells in collagen gels
For embedding cells in collagen gel, suspend the cells in medium at 10× their final desired concentration and mix 1 part of the cell suspension with 100 parts of neutralized collagen solution at +2 to +8°C. Pipette an appropriate volume into the culture vessel and incubate at +15 to +25°C or +37°C. Add medium to the cells immediately after the gel has solidified.

The ratio of cell suspension to collagen solution can be varied but this will result in a variation of the gel consistency.

Changes to Previous Version
- Editorial changes.

References

*available from Roche Diagnostics

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