For life science research only.
Not for use in diagnostic procedures.

Collagen
from rat tail tendon

Version: 18
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Cat. No. 11 179 179 001	30 mg 3 x 10 mg

Store lyophilizate at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Collagen (rat tail)	▪ Lyophilized, cell-culture grade. ▪ Free of microorganisms as tested using an established microbiological enumeration test.	3 vials, 10 mg each

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the lyophilizate is stable through the expiration date printed on the label.

Vial / Bottle	Label	Storage
1	Collagen (rat tail)	Store at +2 to +8°C.

Storage Conditions (Working Solution)

Store the reconstituted solution in sterile acetic acid at +2 to + 8°C.

Reconstitution

Application	Reconstitution
Coating of cell culture dishes	Dissolve the lyophilizate with 5 ml sterile 0.2% acetic acid (v/v) to a final concentration of 2 mg/ml. *For coating culture dishes the final concentration should be 1 to 2 mg/ml. The product can be used in cell culture without additional filtration when stored in sterile 0.2% acetic acid (v/v).*
Preparation of collagen gels	Dissolve the lyophilizate in 3.3 ml sterile 0.2% acetic acid (v/v) to a final concentration of 3 mg/ml.

⚠ ***Do not stir when adding acetic acid to the lyophilizate. Pour the acetic acid onto the lyophilizate and let it stand for several hours until it has dissolved. For total dissolution, it may be necessary to incubate the bottle for up to a maximum of 24 hours at +15 to +25°C.***

1.3. Additional Equipment and Reagent required

For coating cell culture dishes

- Sterile, 0.2% acetic acid (v/v)
- Medium or buffer for washing

For preparation of collagen gels – Method 1

- Sterile, 0.2% acetic acid(v/v), pH 3.0 or 1 mM hydrochloric acid solution, pH 3.0
- Phenol red (optional)
- 25% ammonia solution (v/v)
- Cell culture medium
- 35 mm petri dish
- 60 mm petri dish

Preparation of collagen gels – Method 2

- Sterile, 0.2% acetic acid (v/v), pH 3.0 or 1 mM hydrochloric acid, pH 3.0
- Sterile, 10x- or 5x-concentrated medium, with sodium bicarbonate, pH 7.4
  Read the instructions provided by the supplier of the 10x- or 5x-concentrated medium regarding the appropriate amount of sodium bicarbonate for the specific medium used.
- Sterile, 10x 0.2 M HEPES, pH 7.3: Dilute 1 M HEPES tissue culture tested 1:5 with sterile, double-distilled water.

For subculturing of cells

- Collagenase, such as Collagenase A*, 0.1% in HBSS
- Trypsin/EDTA or Dispase* (optional)

1.4. Application

Rat tail collagen is useful for cultivating cells which need a substrate to grow and to proliferate. It can be used for the:
- Coating of surfaces (culture vessels, slides, cover slips, etc.).
- Preparation of collagen gels.

2. How to Use this Product

2.1. Protocols

Coating cell culture dishes with collagen

Collagen as a substrate is used in the form of either a thin film of dried collagen or a hydrated collagen gel. A thin film of dried collagen is prepared by spreading the collagen solution onto the surface of a dish and air dried.
See section, **Reconstitution** *for additional information.*

1. Pipette 2.5 µl of reconstituted lyophilizate (2 mg/ml) per 1 cm^2 surface area to be coated (5 µg/cm^2).
   This can be increased or decreased to fit the application.
2. Carefully spread the collagen solution with a sterile rubber policeman on the bottom of the culture dish.
3. Air dry in the laminar flow hood for approximately 60 minutes at +15 to +25°C.
4. If desired, wash the coated surface with medium or buffer (optional).
5. Use the dishes immediately or store under sterile conditions.

Preparation of collagen gels

Collagen gels can be prepared by a number of different procedures.
- Method 1: Expose ammonia vapor to the collagen solution.
- Method 2: Adjust the pH and ionic strength of the collagen solution.

For the three dimensional culture of various cell types, the rat tail collagen gel has proved to be an easy and useful system.

⚠ ***Always work under a laminar flow hood.***

Method 1

1. 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 to a final concentration of 3 mg/ml.
   – If 2 µl phenol red is added, the change of pH is easier to control.
2. Let stand overnight to swell.
3. Pipette 100 µl of this collagen solution per 1 cm^2 surface area to be covered into the culture vessel.
   – This gives an approximately 1 mm thick collagen gel layer.
4. Expose the collagen solution to ammonia vapors at +15 to +25°C or +37°C.
5. For a collagen gel in a 35 mm petri dish, pipette 100 µl 25% ammonia solution (v/v) into a 60 mm petri dish.
   – Place a 35 mm dish containing 1 ml collagen solution into the 60 mm dish and close.
   If phenol red is present in the collagen solution, the change of the pH can be easily observed (color change to neutral).
   – Remove the 35 mm dish after a maximum of 2 minutes if there is no pH indicator in the collagen solution.
   If the gel layer is thicker, the gel needs longer to solidify.
6. The gel should be solidified after approximately 2 minutes.
   – Equilibrate the gel with an appropriate amount of medium for 30 minutes to remove excess ammonia which is toxic to the cells.
   A longer period for equilibration is needed for a thicker collagen layer.

7 Aspirate the medium.

8 Seed the cells onto the gel.

Method 2

1 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 to a final concentration of 3 mg/ml.

2 Mix together the following solutions at +2 to +8°C, avoiding the formation of air bubbles:

Solution	Contents
1	1 part sterile, 10x-concentrated medium with sodium bicarbonate, pH 7.4.
2	1 part sterile, 10x 0.2 M HEPES, pH 7.3 (Dilute 1 M HEPES 1:5 with sterile, double-distilled water).
3	8 parts sterile collagen solution.

– Alternatively, mix together the following solutions at +2 to +8°C, avoiding the formation of air bubbles:

Solution	Contents
1	2 parts sterile, 5x-concentrated medium with sodium bicarbonate, pH 7.4.
2	1 part sterile, 10x 0.2 M HEPES, pH 7.3 (Dilute 1 M HEPES 1:5 with sterile, double-distilled water).
3	7 parts sterile collagen solution.

– The mixture remains liquid at +2 to +8°C.

3 Pipette 100 µl of this neutralized collagen solution per 1 cm^2 surface area to be covered into the culture vessel.
– This gives an approximately 1 mm thick collagen gel layer.

4 Incubate for approximately 2 to 3 hours at +15 to +25°C or at +37°C in a humidified atmosphere to allow gel formation.
– Store the culture dishes at +2 to +8°C under sterile conditions.

5 Cover the gel with medium for approximately 15 minutes for equilibration before inoculating the cell suspension.

6 Plate the cells as usual.
To allow the formation of a homogeneous gel and to avoid clump formation, do not or very carefully move culture vessels during gel formation.

Subculture of cells

1 Digest the collagen with collagenase, such as Collagenase A*, 0.1% in HBSS for approximately 10 to 20 minutes or until the gel is digested at +37°C.

2 Recover the cells and wash 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

1. Suspend the cells in medium at 100x their final desired concentration.
2. Mix 1 part of the cell suspension with 100 parts of neutralized collagen solution at +2 to + 8°C.
3. Pipette an appropriate volume into the culture vessel and incubate at +15 to +25°C or +37°C.
4. Add medium to the cells immediately after the gel has solidified.
   The ratio of cell suspension to collagen solution can be varied, however this will result in a variation of the gel consistency.

2.2. Parameters

Biological Activity

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

Specificity

Species specificity: Active on most vertebrate cells.

Working Concentration

Coating of cell culture vessels: 5 µg/cm^2
Preparation of collagen gels: 2 to 3 mg/ml final concentration.

3. Additional Information on this Product

3.1. Test Principle

Preparation

Collagen is purified from rat tail tendon and consists primarily of type I collagen.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Dispase® I (neutral protease, grade I)	10 x approx. 2 mg, ≥ 20 U	04 942 086 001
Dispase® II (neutral protease, grade II)	5 x 1 g	04 942 078 001
Collagenases	Collagenase A, 100 mg	10 103 578 001
	Collagenase B, 100 mg	11 088 807 001
	Collagenase D, 100 mg	11 088 858 001
	Collagenase A, 500 mg, *Not available in US*	10 103 586 001
	Collagenase B, 500 mg, *Not available in US*	11 088 815 001
	Collagenase D, 500 mg, *Not available in US*	11 088 866 001
	Collagenase A, 2.5 g	11 088 793 001
	Collagenase B, 2.5 g	11 088 831 001
	Collagenase D, 2.5 g	11 088 882 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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