

3DCultr Collagen, Type I, from mouse tail

Product description

Collagen is the primary component of the extracellular matrix in connective tissues and visceral organs, with the widest distribution in the skin, tendons, and bones. Structurally and genetically, collagen is classified into different types. Type I collagen, for instance, is composed of two $\alpha 1$ chains and one $\alpha 2$ chain, forming a heterotrimer approximately 300 nm in length. When used as a gel, collagen aids in the in vitro culture and enhances the expression of cell-specific morphology and functions. Collagen can also be utilized as a thin layer to promote cell attachment, with applications including the study of tumor cell invasion and migration, monocytic cell culture or differentiation research, and the radioautography of granulocytes and macrophages. Type I collagen is also employed in studies aimed at maintaining liver cell function, elevating differentiation status, and increasing the transcriptional levels of hepatic cell genes. This product is sourced from the mouse tail and is dissolved in 0.02 M HAc solution. Its purity, determined by SDS-PAGE, exceeds 90%, and it is sterile.

Specifications

Catalog Number	C231102E/C231102S
Specifications	2 mL/10 mL

Storage

Store at 4°C, valid for 1 year. Do not freeze.

Notes

1. For your safety and health, please wear a lab coat and disposable gloves.
2. Please perform the entire operation on ice, because collagen I can quickly gel at room temperature.
3. Please perform the entire operation aseptically in a sterile environment to avoid contamination that may affect cell growth.
4. This product is for scientific research purposes only.

Instructions

Collagen I can be gelled onto coverslips or tissue culture dishes or used as a thin film coating for cell attachment. Cells can be cultured on top of the gel, within the gel, or between gel layers.

Thin coating procedure

[Note]The recommended coating concentration is 5-10 $\mu\text{g}/\text{cm}^2$. with a preferred concentration of 5 $\mu\text{g}/\text{cm}^2$. It is suggested to optimize the concentration based on specific cell culture systems.

1. Dilute Collagen Type I to a concentration of 50 $\mu\text{g}/\text{mL}$ with 0.02 M acetic acid. Collagen Type I is not soluble in neutral pH solutions.
2. Coat the culture dish with 5 $\mu\text{g}/\text{cm}^2$ of Collagen Type I. For example, the surface area of a 35 mm culture dish is approximately 10 cm^2 , so 1-2 mL of working solution is sufficient to cover the dish.
3. Incubate at room temperature for 1 hour.
4. Carefully aspirate the remaining solution and wash with PBS or serum-free culture medium to remove excess acetic acid.
5. The coated culture dish can be used immediately or air-dried. It can be stored at 2~8°C under sterile conditions for up to one week.

Gelling procedure

Collagen I gels when its pH reaches alkaline by following steps:

1. Prepare an ammonia vapor chamber by placing a sterile gauze sponge, approximately 5 cm in size, on the lid of a 150 mm culture dish. Saturate the gauze with ammonium hydroxide and place the lid on the 150 mm culture dish. Set aside.
2. Evenly apply collagen I on the surface to be coated. The thickness can be changed as needed. Collagen I (50-100 μL) is sufficient to coat a 22 mm coverslip. For 100 mm diameter Petri dishes, add approximately 6 mL each; for 60 mm Petri dishes, add approximately 2.3 mL, and for 35 mm Petri dishes, add approximately 1 mL.
3. Transfer the collagen-coated coverslip or Petri dish with a lid to the ammonia vapor chamber and expose for three minutes.
4. Soak the coated coverslip or Petri dish in sterile distilled water (add 5 mL to a 35 mm Petri dish, add 10 mL to a 60 mm Petri dish, etc.) for 30 minutes. Aspirate the original distilled water and add 0.5-1.0 mL sterile distilled water, and place it in a laminar flow hood overnight.
5. Aspirate the distilled water, replace with serum-containing balanced salt buffer solution and store at 2~8°C.

Alternate gelling procedure

1. Place the following items on ice: Collagen I, sterile 10× PBS, sterile distilled water, sterile 1M NaOH.
2. Determine the volume of Collagen Type I solution needed to achieve the desired final concentration.

3. Place sterile tubes on ice for storing Collagen Type I.
4. Perform the following steps under sterile conditions.
 - 1) Add 10×PBS (final volume/10) mL

Calculate the volume of Collagen I to use (do not add to tube until step 4.6)

$$\frac{\text{Final volume} \times \text{final collagen I concentration (mg/mL)}}{\text{Specific concentration on bottle label (see specific batch number)}} = \text{Amount of collagen to be added}$$

- 2) Add (volume of collagen to be added × 0.023) mL of sterile ice-cold 1M NaOH to the 10 × PBS solution.
- 3) Add the following volume of sterile ice-cold distilled water to the solution in 4.3:
Add volume of distilled water = V (final) - V (collagen) - V (10×PBS) - V (1M NaOH)
- 4) Mix the contents of the tube and place in ice.
- 5) Add collagen I and calculate the volume, mix well, and keep on ice until ready.
5. The Collagen Type I solution can be used immediately or kept on ice for 2-3 hours.
6. When ready to use, add the solution to the cell culture device under sterile conditions and gel at 37°C for 30 minutes.