gibco
by life technologies

Collagen I, Bovine

Description

Collagen is a fibrous protein that consists of three α-chains which can combine to form a rope-like triple helix, providing tensile strength to the extracellular matrix (ECM) where it plays a key role in cell growth, differentiation, attachment, and cell migration. The α-chains contain GXY repeats: glycine (G) is a small amino acid that fits well in the triple helix. X and Y are typically proline and hydroxyproline, which are critical for collagen stability. Type I is the most common fibrillar collagen (90%), and is mostly found in skin, bone, tendons, and other connective tissues. Bovine collagen I can be prepared as a clear gel providing a 3D matrix or surface coated on tissue culture plates as a substrate for culturing primary cells such as keratinocytes and hepatocytes. Collagen I (Bovine) solution is supplied at 5 mg⁄mL providing the flexibility to dilute to lower concentrations.

Product	Catalog No.	Amount	Storage	Shelf Life*
Collagen I, Bovine	A10644-01	10 mL	2°C to 8°C; Protect from light	12 months

* Shelf Life duration is determined from Date of Manufacture.

Product Use

For Research Use Only. Not for use in diagnostic procedures.

Important Information

- Do not freeze.
- Perform manipulations on ice (2°C to 8°C) as gelling may occur rapidly at room temperature.
- We recommend that the following procedures be performed in an aseptic environment using aseptic techniques to prevent contamination.

Safety Information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Use – Gelling Procedures

1. Place collagen (5mg/mL), sterile 10X phosphate buffered saline (PBS) or 10X Medium 199 (M199), sterile distilled water (dH2O), and sterile 1N NaOH on ice.
2. Determine the concentration and final volume of collagen needed for experimentation. See **Example Calculation**. A concentration of 3–4 mg/mL is recommended for optimal gel formation.
3. Determine the amount of reagents needed so that collagen is at the desired concentration in 1X PBS or M199 with normal osmolality and neutral pH. See **Example Calculation**.

Example Calculation

V_t = Total volume of collagen gel desired

Volume of collagen needed (V1) =

$$\frac{\text{(Final conc. of collagen)} \times \text{(Total Volume } (V_t))}{\text{Initial conc. of collagen}}$$

Volume of 10X PBS needed (V2) =

$$\frac{\text{Total Volume } (V_t)}{10}$$

Volume of 1N NaOH needed (V3) = (V1) × 0.025

Volume of dH_2O needed (V4) = (V_t) – (V1 + V2 + V3)

Example Calculation, continued

Example calculation for a 3 mg/mL firm gel at a total volume of 10 mL, as follows:

V_t =10 mL

$$V1 = \frac{(3\ mg/mL)\ (10\ mL)}{(5\frac{mg}{mL})} = 6\ \text{mL}$$

$$V2 = \frac{10\ mL}{10} = 1\ \text{mL}$$

V3= (6 mL) (0.025)= 0.15 mL

V4= 10 mL – (6 mL + 1 mL + 0.15 mL) = 2.85 mL

4. In a sterile tube mix the dH_2O, 1N NaOH, and 10X PBS.
5. Slowly pipet the collagen into the tube, and gently pipet solution up and down to mix well. The resulting mixture should achieve a pH of 6.5–7.5 (optimal pH is 7.0).
6. Immediately dispense the collagen into the desired culture vessels or store collagen solution on ice. Gelling may occur rapidly at room temperature.
7. Incubate at 37°C in humidified incubator for 30–40 minutes or until a firm gel is formed.
8. Rinse the gel with sterile 1X PBS or cell culture medium before seeding cells.

Thin Coating Procedure

Note: Optimization for desired protein concentration may be required. A starting concentration of 5 µg/cm² is recommended. Further dilution may be desired depending on the cell system.

1. Determine the volume needed for experimentation.
2. Dilute the collagen to 50 µg/mL in 20 mM acetic acid at the final volume needed:

Volume of collagen (V1)=

$$\frac{\text{(50 µg/mL of collagen)} \times \text{(Final Volume)}}{\text{(Initial Concentration of collagen (µg/mL))}}$$

Volume of 20 mM acetic acid=

Final Volume – Volume of collagen (V1)

3. Add solution to plates or dishes at 5 µg/cm² (e.g., 50 µg, or 1 mL of 50 µg/mL collagen is required for coating a 35-mm dish, which has a surface area of ≈10 cm²). Further dilution may be desirable for cell cultures requiring lower cell-surface adhesion strengths.
4. Incubate at room temperature for 1 hour.
5. Carefully aspirate solution from the well or dish.
6. Rinse dish three times with equal volumes of sterile 1X PBS or media to remove the acid.
7. Plates may be used immediately or air dried (stored at 2°C to 8°C) for future use.

Related Products

Product	Catalog No.
PBS, pH 7.2	20012
PBS, 10X, pH 7.2	70013
DPBS, without calcium and magnesium	14190
DPBS, 10X, no calcium, no magnesium	14200
Medium 199	11150
Medium 199, 10X	11825
HBSS, calcium, magnesium, no phenol red	14025
AlgiMatrix® 3D Culture System	12684
Collagen I, Rat Tail	A10483
Water, Distilled	15230

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

				
Use By:	Manufacturer	Batch code	Keep away from light	Temperature Limitation
				
Catalog number	Consult instructions for use	Caution, consult accompanying documents	Sterilized using aseptic processing techniques	

Limited Product Warranty

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For further assistance, email **techsupport@lifetech.com**

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