GIBCO
invitrogen cell culture

Collagen I, Rat Tail

For Cell Culture

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 extracelluar matrix (ECM). The α chains contain GXY repeats: glycine (G) is a small amino acid that fits well the triple helix. X and Y are typically proline and hydroxyproline, which is 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.

Description	Cat. No.	Size
Collagen I, Rat Tail	A10483-01	20 mL

Intended Use

For research use only. CAUTION: Not intended for human or animal diagnostic or therapeutic uses.

Storage

Store in the dark at 2 to 8°C.

Shelf Life

12 months from date of manufacture.

Source

Rat tail tendons

Concentration

5 mg/mL

Precautions

Do not Freeze

Use: Gelling Procedures

Note: It is recommended that the following procedures be performed in an aseptic environment using aseptic techniques to prevent contamination.

1. Place collagen (5mg/mL) , sterile 10X phosphate buffered saline (PBS) or 10X Medium 199 (M199), sterile distilled water (dH_2O) & sterile 1N NaOH on ice.
2. Determine the concentration and final volume of collagen needed for experimentation. A concentration of 3 or 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.

V *= Total volume of collagen gel desired*

Volume of collagen needed (V1) =

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

Volume of 10X PBS needed (V2) =

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

Volume of 1N NaOH needed (V3) =

(Final volume of collagen needed (V1)) x 0.025

Volume of dH_2O needed (V4) =

Total Volume (V) - (V1 + V2 + V3)

Example: A requirement for a 4 mg/mL firm gel at a total volume of 10 mL, can be calculated as follows:

V *=10 mL*

$$\textbf{\textit{V1}} = \frac{\text{(4 mg/mL) (10 mL)}}{\text{(5 mg/mL)}} = \textbf{8 mL}$$

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

V3*= (8 mL) (0.025)* ***= 0.20 mL***

V4*= 10 mL- (8mL +1mL +0.2 mL)* ***= 0.8 mL***

4. In a sterile tube mix the dH_2O, 1N NaOH, and 10X PBS.
5. Slowly pipette the collagen to the tube, and gently pipette 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. Place the collagen into the desired plates or dishes immediately or store them on ice. Gelling may occur rapidly at room temperature.
7. Incubate in a 37°C, 95% humidity incubator for 30-40 minutes or until a firm gel is formed.
8. Rinse the gel with 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 per cm^2 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 0.02 M acetic acid at the final volume needed:

Volume of collagen (V1)=

(50 µg/mL of collagen) x (Final Volume)
*(Initial Concentration of collagen (*µg/mL *))*

Volume of 0.02 M acetic acid=

Final Volume - Volume of collagen (V1)

3. Add solution to plates or dishes at 5 µg per cm^2. Further dilution may be desirable for cell cultures requiring lower cell-surface adhesion strengths. (e.g. 50 ug, or 1 mL of 50 µg/mL of collagen is required for coating a 35 mm dish, which has a surface area of approximately 10 cm^2).
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 PBS or media to remove the acid.
7. Plates may be used immediately or air dried (stored at 2 to 8°C) for future use.

Related Products:

Geltrex™ Reduced Growth Factor Basement Membrane Matrix, (12760)

AlgiMatrix™ 3D Culture System, (12684)

Dulbecco's Phosphate Buffered Saline (DPBS) (10X), liquid (14200)

Medium 199 (10X), liquid (11825)

Water, distilled, (15230)

Contacts

For further information on this or other GIBCO® products, contact Technical Services at the following:

United States TECH-LINE SM : 1 800 955 6288

Canada TECH-LINE: 1 800 757 8257

Outside the U.S. and Canada, refer to the GIBCO products catalogue for the TECH-LINE in your region.

You may also contact your Invitrogen Sales Representative or our World Wide Web site at www.invitrogen.com.

References

1. Chen, S., R. Revoltella, S. Papini, M. Michelini, W. Fitzgerald, J. Zimmerberg, and L. Margolis. 2003.Multilineage differentiation of rhesus monkey embryonic stem cells in three-dimensional culture systems. Stem Cells. **21**:281-295.
2. Kokenyesi, R., K. Murray, A. Benshushan, E. Huntley, and M. Kao.2003. Invasion of interstitial matrix by a novel cell line from primary peritoneal carcinosarcoma, and by established ovarian carcinoma cell lines: role of cell-matrix adhesion molecules, proteinases and E-cadherin expression. Gynecol Oncol. **89**:60-72.
3. Kutznetsova, N., S. Chi, and S. Leikin. 1998. Sugars and polyols inhibit fibrillogenesis of type I collagen by disrupting hydrogen-bonded water bridges between the helices. Biochem. **37**:11888-11895.
4. Kutznetsova, N., and S. Leikin. 1999. Does the triple helical domain of type I collagen encode molecular recognition and fiber assembly while telopeptides serve as catalytic domains. J. Bio. Chem. **274**:36083-36088.
5. Leikin, S., D. Rau, and V. Parsegian. 1994. Direct measurement of forces between self-assembled proteins: Temperature-dependent exponential forces between collagen triple helices. Proc. Natl. Acad. Sci. USA. **91**:276-280.
6. Leikina, E., M. Mertts, N. Kuznetsova, and S. Leikin. 2002. Type I collagen is thermally unstable at body temperature. Proc. Natl. Acad. Sci. USA. **99**:1314-1318.
7. O' Shaughnessy, T., H. Lin, and W. Ma. 2003. Functional synapse formation among rat cortical neurons grown on three-dimensional collagen gels. Neuroscience Letters. **340**:169 - 172.
8. Park, D., D. Choi, H. Ryu, H. Kwon, H. Joo, and C. Min. 2003. A well-defined in vitro three-dimensional culture of human endometrium and its applicability to endometrial cancer invasion. Cancer Letters. **195**:185-192.
9. Ritty, T., and J. Herzog. 2003. Tendon cells produce gelatinases in response to type I collagen attachment. J. Ortho. Res. **21**:442-450.
10.Van Oostveldt, K., M. Paape, and C. Burvemich. 2002. Apoptosis of bovine neutrophils following diapedesis through a monolayer of endothelial and mammary epithelial cells. J. Dairy Sci. Ass. **85**:139-147.

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