Cultrex™ 3-D Culture Matrix Rat Collagen I

Catalog Number: 3447-020-01 (100 mg)

PRODUCT DESCRIPTION

Type I Collagen is the major structural component of extracellular matrices found in connective tissue and internal organs, but is most prevalent in the dermis, tendons, and bone. It is a 300 kDa molecule composed of two alpha$_1$(I) chains and one alpha$_2$(I) chain that spontaneously forms a triple helix scaffold at a neutral pH and 37 °C. This phenomenon can be exploited to promote cell attachment, growth, differentiation, migration, and tissue morphogenesis during development.

3-D culture is an innovative approach to modeling the morphological effects of early oncogenesis on three-dimensional microenvironments. When healthy, differentiating cells exhibit a structured, polarized morphology that is critical for cellular formation and function. During carcinoma development, cell cycle controls associated with cellular development, proliferation and death are lost, and as a result, these structures are disrupted. In effect, the morphology of these structures can be used as a measure to study factors in early carcinoma development. The 3-D Culture Matrix Rat Collagen was designed to be used as a gel on which to grow cells or a media additive alone or in concert with other basement membrane components to study cellular growth and differentiation in three dimensions *in vitro*.

INTENDED USE

Cultrex 3-D Culture Matrix Rat Collagen I may be used as a hydrogel or thin coating for tissue culture vessels. It may also be used to supplement customized coatings, hydrogels or medium formulations for cell culture. To provide the most standardized Collagen I for use in 3-D cultures, a special process is employed to provide material at a standard concentration of approximately 5 mg/mL. This material is then incorporated in a 3-D culture to validate efficacy.

PRODUCT SPECIFICATIONS

Concentration	5 mg/mL
Source	Rat tail tendons
Storage Buffer	20 mM Acetic Acid
Stability	Product is stable for a minimum of 3 months if stored at 2-8 °C. See lot specific Certificate of Analysis for expiration date.
Storage	Store at 2-8 °C. **Do Not Freeze.**

PRECAUTION

When handling bio-hazardous materials such as human cells, safe laboratory procedures should be followed and protective clothing should be worn.

LIMITATIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- Results may vary due to variations among tissue/cells derived from different donors or sources.

Global bio-techne.com info@bio-techne.com TEL +1 612 379 2956 **USA** TEL 800 343 7475 **Canada** TEL 855 668 8722
Europe | Middle East | Africa TEL +44 (0)1235 529449 **China** TEL +86 (21) 52380373

MATERIAL QUALIFICATIONS

Sterility Testing:

- No bacterial or fungal growth detected after a 14 day culture.
- No mycoplasma contamination detected by PCR.
- Endotoxin concentrations < 20 EU/mL by LAL assay.

Gelling Assay:

- Cultrex 3-D Culture Matrix Rat Collagen I forms a firm gel at neutral pH and 37 °C when diluted to 0.4 mg/mL.

Functional Assays:

- Cell Attachment: Tested for the ability to promote cell attachment and spreading of HT-1080 human fibrosarcoma cells.
- 3-D Culture: Cultrex 3-D Culture Matrix Rat Collagen I promotes attachment and growth of murine endothelial SVEC4-10 cells.

GELLING PROCEDURE

To prevent contamination maintain aseptic techniques in a laminar flow biological hood throughout this procedure. Working with solutions that are pre-chilled at 2-8 °C, and keeping solutions on ice extends the time that Cultrex 3-D Culture Matrix Rat Collagen I will remain in solution after neutralization.
Note: *The recommended working concentration for Cultrex 3-D Culture Matrix Rat Collagen I 1 mg/mL.*

Gelling Procedure using 1X PBS and 1 N NaOH for Neutralization.

1. Place the following on ice:
   a. Cultrex 3-D Culture Matrix Rat Collagen I (5 mg/mL)
   b. Sterile 10X Phosphate Buffered Saline (PBS)
   c. Sterile distilled water
   d. Sterile 1N NaOH (fresh)
2. Determine the concentration and final volume of Collagen needed for experimentation.
   a. Volume of Collagen needed =

$$\frac{\text{(Final concentration of Collagen) x (Total volume)}}{\text{Initial concentration of Collagen}}$$

   b. Volume of 10X PBS needed =

$$\frac{\text{Total Volume}}{10}$$

   c. Volume of 1N NaOH needed = **[(Volume of Collagen I, step a) x 0.023 mL]**
   d. Volume of distilled water needed = **[Total Volume - (sum of volumes from steps a+b+c)]**
3. In a sterile tube mix the 10X PBS, and distilled water, and 1N NaOH.
4. Add the Cultrex 3-D Culture Matrix Rat Collagen I to the tube and pipette up and down to mix. **Do not vortex.**
5. Place the diluted Cultrex 3-D Culture Matrix Rat Collagen I into the desired plates or dishes. This solution is stable for up to 1 hour on ice.
   Note: *Plates may be centrifuged 300 x g for 10 minutes at 2-8 °C to prevent bubbles from forming in the gel.*
6. Incubate the plate at 37 °C for 1 hour to promote gel formation.

GELLING PROCEDURE *Continued*

Gelling Procedure using 7.5% (w/v) Sodium Bicarbonate for Neutralization.

1. Place the following on ice:
   a. Cultrex 3-D Culture Matrix Rat Collagen I (5 mg/mL)
   b. Sterile 10X PBS
   c. Sterile distilled water
   d. Sterile 7.5% Sodium Bicarbonate
2. Determine the concentration and final volume of Cultrex 3-D Culture Matrix Rat Collagen I (5 mg/mL) needed for experimentation.
   a. Volume of Collagen needed =

$$\frac{\text{(Final concentration of Collagen) x (Total volume)}}{\text{Initial concentration of Collagen I}}$$

   b. Volume of 10X PBS needed =

$$\frac{\text{Total Volume}}{10}$$

   c. Volume of 7.5% Sodium Bicarbonate needed = [**(Volume of Collagen I, step a) x 0.0125 mL**]
   d. Volume of distilled water needed = [**Total Volume - (sum of volumes from steps a+b+c)**]
3. In a sterile tube mix the 10X PBS, distilled water, and 7.5% Sodium Bicarbonate.
4. Add the Cultrex 3-D Culture Matrix Rat Collagen I to the tube and pipette up and down to mix. **Do not vortex.**
5. Place the diluted Cultrex 3-D Culture Matrix Rat Collagen I into the desired plates or dishes. This solution is stable for up to 1 hour on ice.
   Note: *Plates may be centrifuged 300 x g for 10 minutes at 2-8 °C to prevent bubbles from forming in the gel.*
6. Incubate the plate at 37 °C for 1 hour to promote gel formation.

High Concentration Gelling Procedure

1. Place Cultrex 3-D Culture Matrix Rat Collagen I (5 mg/mL), 7.5% Sodium Bicarbonate solution, sterile tube, and cell culture plate on ice.
2. Add necessary amount of Cultrex 3-D Culture Matrix Rat Collagen I into sterile tube.
3. Add 5 µL of 7.5% Sodium Bicarbonate per 0.1 mL of Cultrex 3-D Culture Matrix Rat Collagen I (5 mg/mL).
4. Pipette Cultrex 3-D Culture Matrix Rat Collagen I up and down to mix. **Do not vortex.**
5. Pipette Cultrex 3-D Culture Matrix Rat Collagen into a cell culture plate.
   Note: *Plate may be centrifuged for 300 x g for 10 minutes at 2-8 °C to prevent bubbles from forming in the gel.*
6. Incubate the plate at 37 °C for 1 hour to promote gel formation.

DATA EXAMPLE

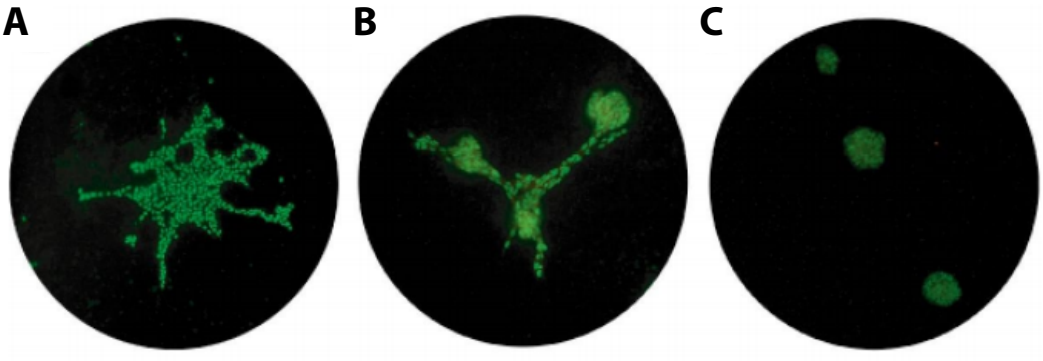

Figure 1: Differentation of MCF-10A mammary epitheliel cells cultured on Cultrex 3-D Culture Matrix Rat Collogen I. Mammary epithelial cells, MCF-10A cultured on 3-D Culture Matrix Collagen I are enduced to differentiate with the addition of 3-D Culture Matrix Laminin I at: A) 0 mg/mL, B) 1 mg/mL, and C) 2 mg/mL.

REFERENCES

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