

CULTREX[®] Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

3-D Culture Matrix™ Rat Collagen I

Catalog #: 3447-020-01

Size: 20 ml

Description: 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. In an attempt at standardization, J. Debnath, *et al.* published guidelines for execution of this assay using MCF-10A mammary epithelial cells as a model.¹ To aid in the advancement of this technology, Trevigen has developed the Cultrex[®] 3-D Culture Matrix™ product line to provide reagents specifically produced for and qualified in 3-D culture studies. The 3-D Culture Matrix™ Collagen I may 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*.

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.

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.

Specifications:

Concentration: Type I Collagen provided at 5 mg/ml (Sircol Assay).

Source: Rat tail tendons

Storage Buffer: 20 mM Acetic Acid

Storage/Stability: Product is stable for a minimum of 3 months if stored at 4°C. **Do Not Freeze.**

Materials Qualification:

Gelling:

- Type I collagen 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: Collagen I promotes attachment and growth of murine endothelial SVEC4-10 cells.

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TREVIGEN[®]

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Sterility Testing:

- No bacterial or fungal growth detected after incubation at 37°C for 14 days following USP XXIV Chapter 71 sterility test.
- No mycoplasma contamination detected by PCR.
- Endotoxin concentrations \leq 20 EU/ml by LAL assay.

Gelling Procedures:

Note: To prevent contamination maintain aseptic techniques in a laminar flow biological hood throughout this procedure. Working with solutions that are pre-chilled at 4°C, and keeping solutions on ice extends the time that collagen I will remain in solution after neutralization.

Material is qualified at 1 mg/mL, and this is the recommended working concentration.

- Place the following on ice:
 - Type I Collagen (5 mg/ml)
 - Sterile 10X PBS
 - Sterile, distilled water (dH₂O)
 - Sterile 1N NaOH (fresh)
- Determine the concentration and final volume of Collagen needed for experimentation.
- Determine the amount of reagents needed so that Collagen I is at the desired concentration in 1X phosphate buffered saline (PBS) neutralized by 1N NaOH:
 - Volume of Collagen needed = $\frac{(\text{Final conc. of Collagen}) \times (\text{Total Volume})}{(\text{Initial conc. of Collagen})}$
 - Volume of 10X PBS needed = $\frac{\text{Total Volume}}{10}$
 - Volume of 1N NaOH needed = (volume of Collagen I) x 0.023 ml
 - Volume of dH₂O needed = Total Volume - (sum of volumes from steps A+B+C)
- In a sterile tube mix the 10X PBS, 1N NaOH and dH₂O.
- Add the Collagen I to the tube and pipette up and down to mix (do not vortex).
- Place the Collagen solution into the desired plates or dishes. Solution is stable for up to one hour on ice. Plates may be centrifuged 300 x g for 10 minutes at 4°C to prevent bubbles from forming in the gel.
- Incubate the plate at 37°C for 1 hour to promote gel formation.

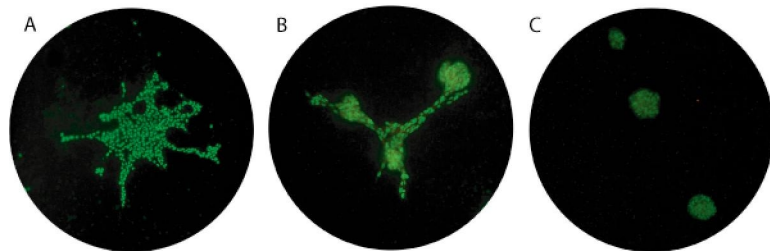
For your cell type, a gelling procedure using 7.5% (w/v) Sodium Bicarbonate for neutralization may be preferred:

- Place the following on ice:
 - Type I Collagen (5 mg/ml)
 - Sterile 10X PBS
 - Sterile, distilled water (dH₂O)
 - 7.5% Sodium Bicarbonate, sterile
- Determine the concentration and final volume of Collagen needed for experimentation.
- Determine the amount of reagents needed so that Collagen I is at the desired concentration in 1X phosphate buffered saline (PBS), neutralized by 7.5% sodium bicarbonate.
 - Volume of Collagen needed = $\frac{(\text{Final conc. of Collagen}) \times (\text{Total Volume})}{(\text{Initial conc. of Collagen I})}$
 - Volume of 10X PBS needed = $\frac{\text{Total Volume}}{10}$
 - Volume of 7.5% sodium bicarbonate needed = (Volume of Collagen I, step a) x 0.0125 ml
 - Volume of dH₂O needed = Total Volume - (sum of volumes from steps A+B+C)

- In a sterile tube mix the 10X PBS, and dH₂O and 7.5% sodium bicarbonate.
- Add the Collagen I to the tube and pipette up and down to mix (do not vortex).
- Place the neutralized Collagen I solution into the desired plates or dishes. This solution is stable for up to 1 hour on ice. Plates may be centrifuged 300 x g for 10 minutes at 4°C to prevent bubbles from forming in the gel.
- Incubate the plate at 37 °C for 1 hour to promote gel formation.

High Concentration Collagen gel method:

- Place Collagen I (5 mg/ml), 7.5% sodium bicarbonate solution, sterile tube and cell culture plate on ice.
- Add necessary amount of Collagen I into sterile tube.
- Add 5 µl of 7.5% sodium bicarbonate per 0.1 ml of Collagen I (5 mg/ml)
- Pipette Collagen I up and down to mix. (Do not vortex.)
- Place neutralized collagen into a cell culture plate. Plate may be centrifuged for 300 x g for 10 minutes at 4 °C to prevent bubbles from forming in the gel.
- Incubate the plate at 37 °C for 1 hour to promote gel formation.



Mammary epithelial cells, MCF-10A cultured on 3-D Culture Matrix™ Collagen I are induced to differentiate with the addition of 3-D Culture Matrix™ Laminin-1 at: a) 0 mg/mL, b) 1 mg/mL, and c) 2 mg/mL.

References:

- Debnath J, Muthuswamy SK, Brugge JS. Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures. *Methods*, 2003 pp. 256-268.
- 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.
- Kokenyesi, R., K. Murray, A. Benschushan, 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.
- 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.
- 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.
- Leikin, S., D. Rau, and V. Parsegian. 1994. Direct measurement of forces between self-assembled proteins: Temperature-dependent forces between collagen triple helices. *Proc. Natl. Acad. Sci. USA*. **91**:276-280.
- Leikina, E., M. Merts, N. Kuznetsova, and S. Leikin. 2002. Type I collagen is thermally unstable at body temperature. *Proc. Natl. Acad. Sci. USA*. **99**:1314-1318.

- 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.
- 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.
- Ritty, T., and J. Herzog. 2003. Tendon cells produce gelatinases in response to type I collagen attachment. *J. Ortho. Res*. **21**:442-450.
- 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.

Related Products:

Catalog#	Description	Size
3415-001-02	Cultrex® Human BME, PathClear®	1 ml
3432-005-02	Cultrex® BME, PathClear®	5 ml
3432-005-01	Cultrex® BME without Phenol Red	5 ml
3431-005-01	Cultrex® BME with Phenol Red; Reduced Growth Factors	5 ml
3433-005-01	Cultrex® BME; no Phenol Red; Reduced Growth Factors	5 ml
3430-005-02	Cultrex® BME with Phenol Red, PathClear®	5 ml
3431-005-02	Cultrex® BME with Phenol Red, Reduced Growth Factor PathClear®	5 ml
3400-010-01	Cultrex® Mouse Laminin I	1 mg
3442-050-01	Cultrex® Bovine Collagen I	50 mg
3410-010-01	Cultrex® Mouse Collagen IV	1 mg
3420-001-01	Cultrex® Human Fibronectin, PathClear®	1 mg
3416-001-01	Cultrex® Bovine Fibronectin, NZHD*	1 mg
3421-001-01	Cultrex® Human Vitronectin, PathClear®	50 µg
3417-001-01	Cultrex® Bovine Vitronectin, NZHD	50 µg
3438-100-01	Cultrex® Poly-L-Lysine	100 ml
3439-100-01	Cultrex® Ploy-D-Lysine	100 ml
3445-048-01	Cultrex® 3-D Culture Matrix™ BME	15 ml
3446-005-01	Cultrex® 3-D Culture Matrix™ Laminin I	5 ml

*New Zealand Herd-Derived



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Storage: 4 °C

(Do Not Freeze)

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