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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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.

1. Place the following on ice:
   a. Type I Collagen (5 mg/ml)
   b. Sterile 10X PBS
   c. Sterile, distilled water (dH_2O)
   d. Sterile 1N NaOH (fresh)
2. Determine the concentration and final volume of Collagen needed for experimentation.
3. 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:
   a. Volume of Collagen needed = $\frac{\text{(Final conc. of Collagen) x (Total Volume)}}{\text{(Initial conc. of Collagen)}}$
   b. Volume of 10X PBS needed = $\frac{\text{Total Volume}}{10}$
   c. Volume of 1N NaOH needed = (volume of Collagen I) x 0.023 ml
   d. Volume of dH_2O needed = Total Volume - (sum of volumes from steps A+B+C)
4. In a sterile tube mix the 10X PBS, 1N NaOH and dH_2O.
5. Add the Collagen I to the tube and pipette up and down to mix (do not vortex).
6. 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.
7. Incubate the plate at 37˚C for 1 hour to promote gel formation.

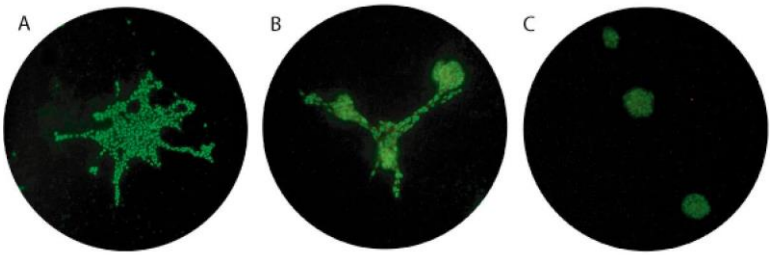
For your cell type, a gelling procedure using 7.5% (w/v) Sodium Bicarbonate for neutralization may be preferred:

1. Place the following on ice:
   a. Type I Collagen (5 mg/ml)
   b. Sterile 10X PBS
   c. Sterile, distilled water (dH_2O)
   d. 7.5% Sodium Bicarbonate, sterile
2. Determine the concentration and final volume of Collagen needed for experimentation.
3. 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.
   a. Volume of Collagen needed= $\frac{\text{(Final conc. of Collagen) x (Total Volume)}}{\text{(Initial conc. of Collagen I)}}$
   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 dH_2O needed = Total Volume - (sum of volumes from steps A+B+C)

4. In a sterile tube mix the 10X PBS, and dH_2O and 7.5% sodium bicarbonate.
5. Add the Collagen I to the tube and pipette up and down to mix (do not vortex).
6. 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.
7. Incubate the plate at 37 °C for 1 hour to promote gel formation.

High Concentration Collagen gel method:

1. Place Collagen I (5 mg/ml), 7.5% sodium bicarbonate solution, sterile tube and cell culture plate on ice.
2. Add necessary amount of Collagen I into sterile tube.
3. Add 5 µl of 7.5% sodium bicarbonate per 0.1 ml of Collagen I (5 mg/ml)
4. Pipette Collagen I up and down to mix. (Do not vortex.)
5. 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.
6. 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 enduced 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:

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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
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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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