For Research Use Only. Not For Use In Diagnostic Procedures.

Cultrex® 3-D Culture Matrix™ Rat Collagen I

Catalog #: 3447-020-01 **Size:** 20 mL **Concentration:** 5 mg/mL

Description: 3-D Culture is an innovative approach to modeling the morphological effects of early oncogenesis on three-dimensional microenvironments, wherein healthy, differentiating cells exhibit a structured, polarized morphology that is critical for tissue 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, the Cultrex 3-D Culture Matrix product line provides 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 utilized 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.

Source: Rat tail tendons

Storage Buffer: 20 mM Acetic Acid

Storage/Stability: Product is stable for up to 3 months from the date of receipt when stored at 2 - 8° C. **Do not freeze.**

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Specifications:

Gelling: Type I Collagen forms a firm gel at neutral pH and 37° C when diluted to 1.0 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 a human epithelial cell line derived from mammary gland (MCF-10A) and human prostate (PC-3). In the presence of assay medium (4 mg/mL BME), these cell lines differentiate into acinar structures.

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 asceptic techniques in a laminar flow biological hood throughout this procedure.

Material is qualified at 2.5 mg/mL, and this is the recommended working concentration.

1. Place the following on ice:
   A. Type I Collagen
   B. Sterile 10X PBS
   C. Sterile distilled water (dH_2O)
   D. Sterile 1 N NaOH (fresh)

2. Determine the concentration and final volume of Collagen I 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), containing 23 mM sodium hydroxide (NaOH).

   A. Volume of collagen needed = $\dfrac{\text{(Final conc. of Collagen) x (Total Volume)}}{\text{Initial conc. of Collagen}}$

   B. Volume of 10X PBS needed = $\dfrac{\text{Total Volume}}{10}$

   C. Volume of 1 N NaOH needed = (Total Volume) x 0.023 mL

   D. Volume of dH_2O needed = Total Volume - (calculated volumes from steps A+B+C)

4. In a clean sterile tube, mix the 10X PBS and 1 N NaOH.

5. Add the dH_2O to the tube and vortex.

6. Add the Collagen I to the tube and pipette up and down to mix (do not vortex).

7. Aliquot into the desired plates or dishes. Solution is stable for 2 - 3 hours on ice.

8. Incubate at 37° C for 1 hour (increase gelling time for lower concentrations).

Concentrated Collagen Method:
This procedure is recommended for experiments that require concentrated Collagen (5 mg/mL).

1. Place the desired volumes of Collagen I into plate wells or dishes.

2. Place the plate or dish into a sterile chamber (any shallow container with lid; large enough for plate/dish to lay flat).

3. Tape a 5 cm^2 gauze sponge or paper towel to the inside of the chamber lid.

4. Saturate the sponge with ammonium hydroxide but not to the point that it will drip into the samples. **Caution: Avoid inhaling noxious ammonium hydroxide fumes.**

5. Uncover the plate or dish, and place the lid containing the sponge on the chamber.

6. Incubate for 5 minutes at 37° C.

7. Remove the plate or dish from the chamber.

8. Place a layer, approximately 1 cm, of sterile PBS or media on top of the gelled collagen. Cover and incubate for 30 minutes.

9. Replace with fresh sterile PBS or media. Cover and incubate overnight in a laminar flow biological hood.

10. Remove the supernate, and culture cells in the desired medium on top of the gelled Collagen I.

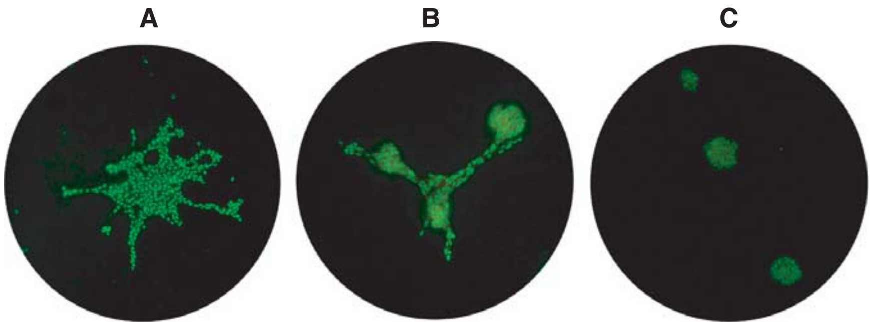

Figure 1: Mammary epithelial cells, MCF-10A, cultured on 3-D Culture Matrix Collagen I are induced to differentiate with the addition of 3-D Culture Matrix Laminin I at A) 0 mg/mL, B) 1 mg/mL, and C) 2 mg/mL.

References:

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R&D Systems

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