CULTREX® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

Cultrex® Rat Collagen I

Catalog: 3440-100-01 **Size:** 20 ml

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.

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. If non-neutralized collagen appears highly viscous, incubate the tube at room temperature for 1 hour, and proceed with neutralization as described (next page).

Functional Assays:

- Tested for the ability to promote cell attachment and spreading of HT-1080 human fibrosarcoma cells.

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 ≤ 20 EU/ml by LAL assay.

Gelling Procedures:

Note: To prevent contamination maintain aseptic techniques in a laminar flow biological hood throughout this procedure. Collagen I gelling at concentrations below 1 mg/mL may be unforgiving regarding neutralization, and as a result, may require optimization.

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Gelling Procedures (cont.):

1. Place the following on ice:
   a. Type I Collagen
   b. Sterile 10X PBS
   c. Sterile, distilled water (dH$_2$O)
   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) x 0.035 ml
   d. Volume of dH$_2$O needed = Total Volume - (calculated volumes from steps A +B+C)
4. In a clean sterile tube mix the 10X PBS and 1N NaOH.
5. Add the dH$_2$O to the tube and vortex.
6. Add the collagen I to the tube and pipet up and down to mix (do not vortex).
7. Place the collagen into the desired plates or dishes. Solution is stable for 2-3 hours on ice.
8. Incubate at 37 °C for 1 hour.

Concentrated collagen method:

This procedure is recommended for experiments that require concentrated collagen (5 mg/ml).

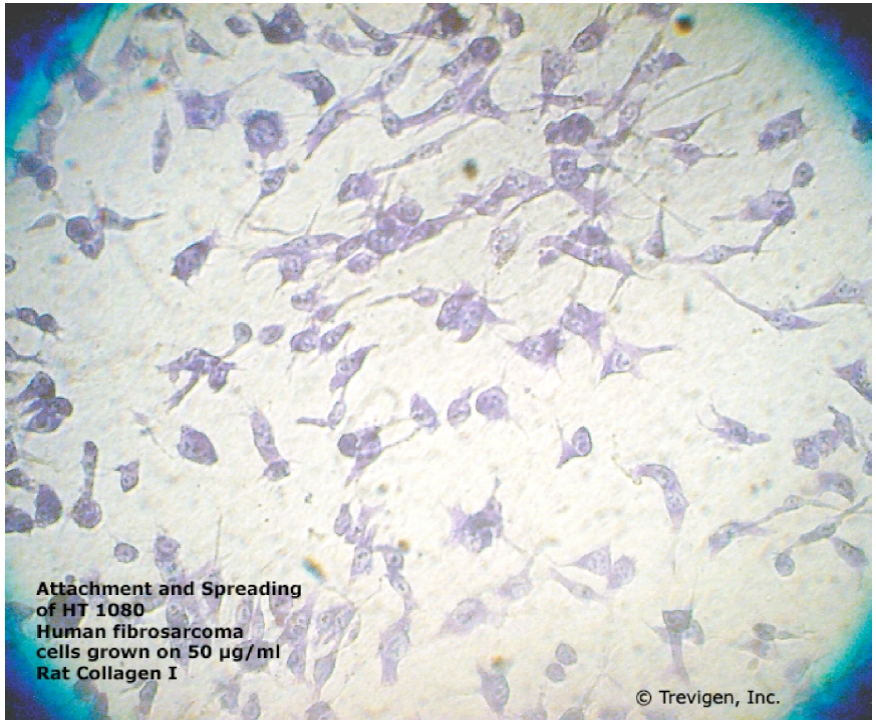
1. Place desired volumes of collagen I into plate wells or dishes.
2. Place the plate or dish into a sterile chamber (shallow container, with lid, large enough for plate/dish to lay flat).
3. Tape a 5 cm^2 gauze sponge or paper-towel to the inside of the chamber lid.
4. Saturate sponge with ammonium hydroxide, but not to the point that it will drip into the samples. **Caution: Avoid inhaling noxious ammonium hydroxide fumes.**
5. Uncover plate or dish, and place the lid containing the sponge on the chamber.
6. Incubate for 5 minutes at 37 °C.
7. Remove plate or petri 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 supernatant, and culture cells in the desired medium on top of the gelled collagen I.

Thin Coating Procedure:

Optimization for desired protein concentration may be required. A starting concentration of 5 µg per cm^2 is recommended.

Thin Coating Procedure (cont.):

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.
   a. Volume of Collagen= $\frac{\text{(50 µg/ml of Collagen) x (Final Volume)}}{\text{(Initial Concentration of Collagen)}}$
   b. Volume of 0.02 M acetic acid= Final Volume - Volume of Collagen (Step A)
3. Add solution to plates or dishes at 5 µg per cm^2. (e.g. 50 µg, 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 for future use.



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