

CULTREX[®] Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

Cultrex[®] Rat Collagen I (LV), Lower Viscosity

Catalog #: 3443-100-01
3443-003-01

Size: 35 ml
1 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 alpha₁(I) chains and one alpha₂(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 is provided at 3 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:

- 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 sterility test guidelines.
- 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. 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.

- Place the following on ice:
 - Type I Collagen (3 mg/ml)
 - Sterile 10X PBS
 - Sterile, distilled water (dH₂O)
 - Sterile 1N NaOH (fresh)

Gelling Procedures (cont.):

- Determine the concentration and final volume of Collagen I 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) 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 pipet up and down to mix (do not vortex).
- Place the Collagen 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.

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 (3 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.

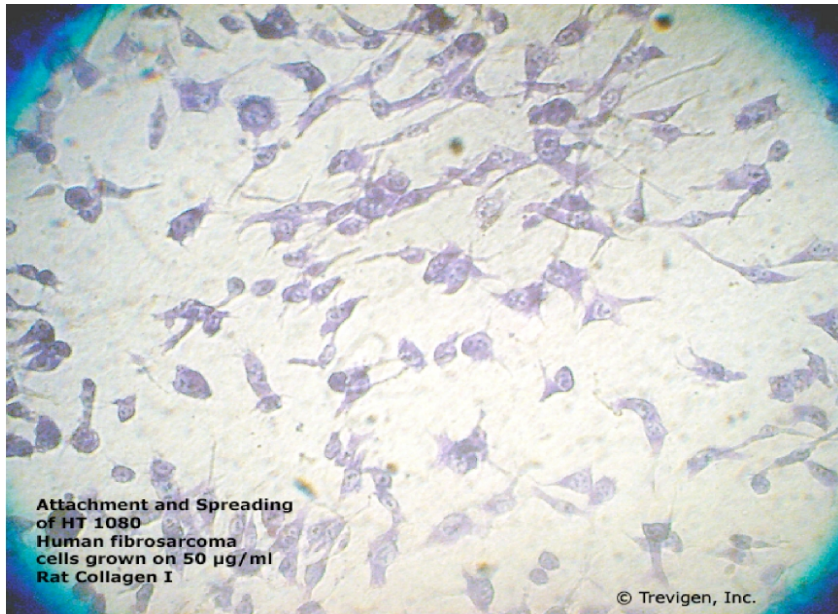
High Concentration Collagen gel method:

- Place Collagen I (3 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 (3 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.

Thin Coating Procedure:

Optimization for desired protein concentration may be required. A starting concentration of 5 µg per cm² is recommended. Increasing the temperature of acidic Collagen I will decrease viscosity. It is recommended that collagen is separated into aliquots prior to warming to maximize shelf life. Aliquots may be warmed to 37°C for up to 5 minutes or 25°C for up to 30 minutes prior to diluting.

- Determine the volume needed for experimentation.
- Dilute the Collagen to 50 µg/ml in 0.02 M acetic acid at the final volume needed.
 - Volume of Collagen = $\frac{(50 \mu\text{g/ml of Collagen}) \times (\text{Final Volume})}{(\text{Initial Concentration of Collagen})}$
 - Volume of 0.02 M acetic acid = Final Volume - Volume of Collagen (Step A)
- Add solution to plates or dishes at 5 µg per cm² (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 cm²).
- Incubate at 37°C for 1 hour.
- Carefully aspirate solution from the well or dish.
- Rinse dish three times with equal volumes of PBS or media to remove the acid.
- Plates may be used immediately or air dried for future use.



References:

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Accessories:

| 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 |
| 3447-020-01 | Cultrex [®] 3-D Culture Matrix [™] Collagen I | 100 mg |

*New Zealand Herd-Derived



**Rat Collagen I, (LV)
Lower Viscosity**

Catalog #: 3443-100-01

Storage: 4 °C

TREVIGEN[®]

1-800-873-8443