# Cultrex® Rat Collagen I, Low Viscosity

**Catalog Numbers:** 3443-003-01 (1 mL)
3443-100-01 (35 mL)

## PRODUCT 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.

## INTENDED USE

Cultrex Rat Collagen I, Low Viscosity is a purified stromal extracellular matrix protein that has been developed, produced, and qualified for general cell culture. Cultrex Rat Collagen I, Low Viscosity is easy to work with and may be used as a hydrogel or thin coating for tissue culture vessels. It may also be used to supplement customized coatings, hydrogels or medium formulations for cell culture.

## PRODUCT SPECIFICATIONS

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|---|---|
| **Concentration** | 3 mg/mL |
| **Source** | Rat tail tendons |
| **Storage Buffer** | 20 mM Acetic Acid |
| **Stability** | Product is stable for a minimum of 3 months if stored at 2-8 °C. |
| **Storage** | Store at 2-8 °C. **Do Not Freeze.** |

## PRECAUTION

When handling bio-hazardous materials such as human cells, safe laboratory procedures should be followed and protective clothing should be worn.

## LIMITATIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- Results may vary due to variations among tissue/cells derived from different donors or sources.

## MATERIAL QUALIFICATIONS

**Sterility Testing:**

- No bacterial or fungal growth detected following 14 days in culture.
- No mycoplasma contamination detected by PCR.
- Endotoxin concentrations ≤ 20 EU/mL by LAL assay.

**Gelling Assay:**

- Cultrex Rat Collagen, Low Viscosity 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.

## GELLING PROCEDURE

To prevent contamination maintain aseptic techniques in a laminar flow biological hood throughout this procedure. Working with solutions that are pre-chilled at 2-8 °C and keeping solutions on ice extends the time that Collagen I will remain in solution after neutralization.

**Gelling Procedure using 1X PBS and 1 N NaOH for Neutralization.**

1. Place the following on ice:
   a. Cultrex Rat Collagen I, Low Viscosity (3 mg/mL)
   b. Sterile 10X Phosphate Buffered Saline (PBS)
   c. Sterile distilled water
   d. Sterile 1N NaOH (fresh)
2. Determine the amount of reagents needed so that Cultrex Rat Collagen I, Low Viscosity is at the desired concentration in 1X PBS neutralized by 1N NaOH.
   a. Volume of Collagen I needed =

$$\frac{\text{(Final concentration of Collagen I) x (Total volume)}}{\text{Initial concentration of Collagen I}}$$

   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 distilled water needed = **[Total Volume - (sum of volumes from steps a+b+c)]**
3. In a sterile tube mix the 10X PBS, 1N NaOH, and distilled water.
4. Add the Cultrex Rat Collagen I, Low Viscosity to the tube and pipette up and down to mix. **Do not vortex.**
5. Place the neutralized Cultrex Rat Collagen I, Low Viscosity into the desired plates or dishes. This solution is stable for up to 1 hour on ice.
   **Note:** *Plates may be centrifuged 300 x g for 10 minutes at 2-8 °C to prevent bubbles from forming in the gel.*
6. Incubate the plate at 37 °C for 1 hour to promote gel formation.

**Gelling Procedure using 7.5% (w/v) Sodium Bicarbonate for Neutralization.**

1. Place the following on ice:
   a. Cultrex Rat Collagen I, Low Viscosity (3 mg/mL)
   b. Sterile 10X PBS
   c. Sterile distilled water
   d. Sterile 7.5% Sodium Bicarbonate
2. Determine the amount of reagents needed so that Cultrex Rat Collagen I, Low Viscosity is at the desired concentration in 1X PBS neutralized by 7.5% Sodium Bicarbonate.
   a. Volume of Collagen needed =

$$\frac{\text{(Final concentration of Collagen I) x (Total volume)}}{\text{Initial concentration of Collagen I}}$$

   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 distilled water needed = **[Total Volume - (sum of volumes from steps a+b+c)]**
3. In a sterile tube mix the 10X PBS, and distilled water, and 7.5% Sodium Bicarbonate.
4. Add the Cultrex Rat Collagen I, Low Viscosity to the tube and pipette up and down to mix. **Do not vortex.**
5. Place the neutralized Cultrex Rat Collagen I, Low Viscosity solution into the desired plates or dishes. This solution is stable for up to 1 hour on ice.
   **Note:** *Plates may be centrifuged 300 x g for 10 minutes at 2-8 °C to prevent bubbles from forming in the gel.*
6. Incubate the plate at 37 °C for 1 hour to promote gel formation.

**High Concentration Gelling Procedure**

1. Place Cultrex Rat Collagen I, Low Viscosity (3 mg/mL), 7.5% sodium bicarbonate solution, sterile tube and cell culture plate on ice.
2. Add necessary amount of Cultrex Rat Collagen I, Low Viscosity into sterile tube.
3. Add 5 µL of 7.5% Sodium Bicarbonate per 0.1 mL of Cultrex Rat Collagen I, Low Viscosity (3 mg/mL).
4. Pipette Cultrex Rat Collagen I, Low Viscosity up and down to mix. **Do not vortex.**
5. Place neutralized Cultrex Rat Collagen I, Low Viscosity into a cell culture plate.
   **Note:** *Plate may be centrifuged for 300 x g for 10 minutes at 2-8 °C to prevent bubbles from forming in the gel.*
6. Incubate the plate at 37 °C for 1 hour to promote gel formation.

**Thin Coat Gelling Procedure**

Optimization for desired protein concentration may be required. A starting concentration of 5 µg per $cm^2$ is recommended. Increasing the temperature of acidic Cultrex Rat Collagen I, Low Viscosity will decrease viscosity. It is recommended that Cultrex Rat Collagen I, Low Viscosity 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.

1. Determine the volume needed for experimentation.
2. Dilute the Cultrex Rat Collagen I, Low Viscosity to 50 µg/mL in 0.02 M acetic acid at the final volume needed.
   a. Volume of Collagen I =

$$\frac{\text{(50 µg/mL of Collagen I) x (Total Volume)}}{\text{Initial concentration of Collagen I}}$$

   b. Volume of 0.02 M acetic acid = Total 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 Cultrex Rat Collagen I, Low Viscosity is required for coating a 35 mm dish, which has a surface area of approximately 10 $cm^2$).
4. Incubate at 37 °C 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.

## REFERENCES


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**Global** bio-techne.com info@bio-techne.com TEL +1 612 379 2956 **USA** TEL 800 343 7475 **Canada** TEL 855 668 8722
**Europe | Middle East | Africa** TEL +44 (0)1235 529449 **China** TEL +86 (21) 52380373