R&D SYSTEMS
a bio-techne brand

Cultrex® Rat Collagen I

Catalog Numbers: 3440-005-01 (5 mg)
3440-100-01 (100 mg)

PRODUCT DESCRIPTION

Type I collagen is the major structural component of extracellular matrices found in connective tissue, internal organs, and most prevalently 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 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

Concentration	5 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. See lot specific Certificate of Analysis for expiration date.
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.

bio-techne®
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

MATERIAL QUALIFICATIONS

Sterility Testing:

- No bacterial or fungal growth detected after a 14 day culture.
- No mycoplasma contamination detected by PCR.
- Endotoxin concentrations < 20 EU/mL by LAL assay.

Gelling Assay:

- Cultrex Rat Collagen I 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 kept on ice extends the time that Cultrex Rat Collagen I will remain in solution.

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

1. Place the following on ice:
   a. Cultrex Rat Collagen (5 mg/mL)
   b. Sterile 10X Phosphate Buffered Saline (PBS)
   c. Sterile distilled water
   d. Sterile 1N NaOH (fresh)
2. Determine the concentration and final volume of Cultrex Rat Collagen I needed for experimentation.
   a. Volume of Collagen needed =

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

   b. Volume of 10X PBS needed =

$$\frac{\text{Total Volume}}{10}$$

   c. Volume of 1N NaOH needed = **[(Volume of Collagen) 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 to the tube and pipet up and down to mix. **Do not vortex.**
5. Pipette the diluted Cultrex Rat Collagen I 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 *Continued*

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

1. Place the following on ice:
   a. Cultrex Rat Collagen I (5 mg/mL)
   b. Sterile 10X PBS
   c. Sterile distilled water
   d. Sterile 7.5% Sodium Bicarbonate
2. Determine the concentration and final volume of Cultrex Rat Collagen I (5 mg/mL) needed for experimentation.
   a. Volume of Collagen needed =

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

   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, 7.5% Sodium Bicarbonate, and distilled water.
4. Add the Cultrex Rat Collagen I to the tube and pipet up and down to mix. **Do not vortex.**
5. Pipette the diluted Cultrex Rat Collagen I 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 (5 mg/mL), 7.5% Sodium Bicarbonate solution, sterile tube, and cell culture plate on ice.
2. Add necessary amount of Cultrex Rat Collagen I into sterile tube.
3. Add 5 µL of 7.5% Sodium Bicarbonate per 0.1 mL of Cultrex Rat Collagen I.
4. Pipette up and down to mix. **Do not vortex.**
5. Pipette Cultrex Rat Collagen I 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.

GELLING PROCEDURE *Continued*

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 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.

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=

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

   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 Cultrex Rat Collagen I 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 1X PBS or media to remove the acid.
7. Plates may be used immediately or air dried for future use.

REFERENCES

1. Chen, S. *et al.* (2003) Stem Cells **21**:281.
2. Kokenyesi, R. *et al.* (2003) Gynecol. Oncol. **89**:60.
3. Kutznetsova, N. *et al.* (1998) Biochem. **37**:11888.
4. Kutznetsova, N. and S. Leikin (1999) J. Bio. Chem. **274**:36083.
5. Leikin, S. *et al.* (1994) Proc. Natl. Acad. Sci. USA **91**:276.
6. Leikina, E. *et al.* (2002) Proc. Natl. Acad. Sci. USA **99**:1314.
7. O' Shaughnessy, T. *et al.* (2003) Neuroscience Letters **340**:169.
8. Park, D. *et al.* (2003) Cancer Letters **195**:185.
9. Ritty, T. and J. Herzog (2003) J. Ortho. Res. **21**:442.
10. Van Oostveldt, K. *et al.* (2002) J. Dairy Sci. Ass. **85**:139.