CULTREX® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

Cultrex® Bovine Collagen I

Catalog: 3442-050-01 **Size:** 50 mg

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 is provided at 5 mg/mL (Sircol Assay).
Source:	Fetal Bovine Extensor 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 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. 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.

1. Place the following on ice:
   a. Type I Collagen (5 mg/ml)
   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 I needed for experimentation.

TREVIGEN®
8405 Helgerman Court, Gaithersburg, MD 20877 USA
Voice: 1-800-TREVIGEN (1-800-873-8443) • 301-216-2800
Fax: 301-560-4973 • e-mail: info@trevigen.com • www.trevigen.com

Gelling Procedures (cont.):

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 0.023 ml
   d. Volume of dH_2O needed = Total Volume - (sum of volumes from steps A+B+C)
4. In a sterile tube mix the 10X PBS, 1N NaOH and dH_2O.
5. Add the Collagen I to the tube and pipet up and down to mix (do not vortex).
6. 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.
7. 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:

1. Place the following on ice:
   a. Type I Collagen (5 mg/ml)
   b. Sterile 10X PBS
   c. Sterile, distilled water (dH_2O)
   d. 7.5% Sodium Bicarbonate, sterile
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 7.5% sodium bicarbonate.
   a. Volume of Collagen needed= $\frac{\text{(Final conc. of Collagen) x (Total Volume)}}{\text{(Initial conc. of Collagen I)}}$
   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 dH_2O needed = Total Volume - (sum of volumes from steps A+B+C)
4. In a sterile tube mix the 10X PBS, and dH_2O and 7.5% sodium bicarbonate.
5. Add the Collagen I to the tube and pipette up and down to mix (do not vortex).
6. 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.
7. Incubate the plate at 37 °C for 1 hour to promote gel formation.

High Concentration Collagen gel method:

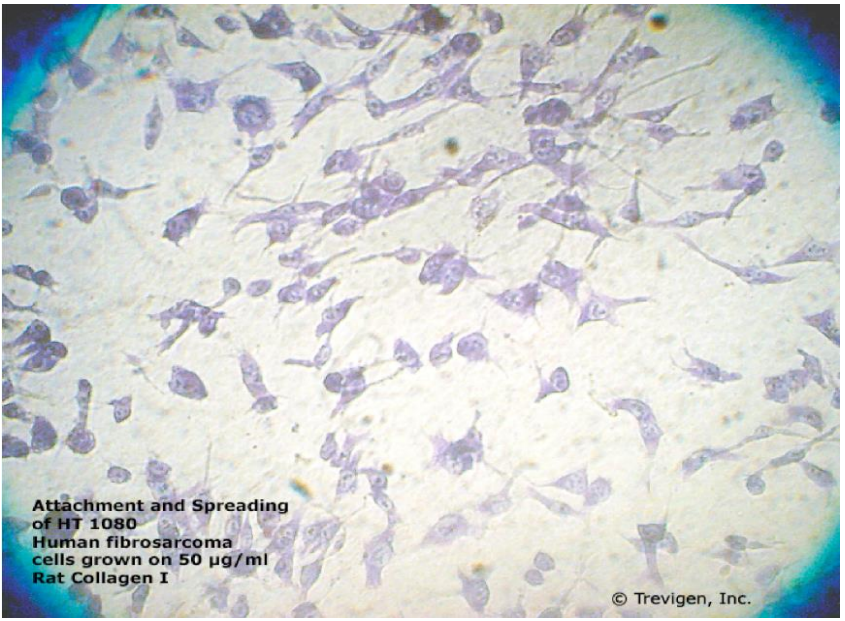
1. Place Collagen I (5 mg/ml), 7.5% sodium bicarbonate solution, sterile tube and cell culture plate on ice.
2. Add necessary amount of Collagen I into sterile tube.
3. Add 5 µl of 7.5% sodium bicarbonate per 0.1 ml of Collagen I (5 mg/ml)
4. Pipette Collagen I up and down to mix. (Do not vortex.)
5. 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.
6. 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 cm^2 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.

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 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:

1. Chen, S., R. Revoltella, S. Papini, M. Michelini, W. Fitzgerald, J. Zimmerberg, and L. Margolis. 2003. Multilineage differentiation of rhesus monkey embryonic stem cells in three-dimensional culture systems. *Stem Cells*. **21**:281-295.
2. Kokenyesi, R., K. Murray, A. Benshushan, E. Huntley, and M. Kao.2003. Invasion of interstitial matrix by a novel cell line from primary peritoneal carcinosarcoma, and by established ovarian carcinoma cell lines: role of cell-matrix adhesion molecules, proteinases and E-cadherin expression. *Gynecol Oncol.* **89**:60-72.
3. Kutznetsova, N., S. Chi, and S. Leikin. 1998. Sugars and polyols inhibit fibrillogenesis of type I collagen by disrupting hydrogen-bonded water bridges between the helices. *Biochem*. **37**:11888-11895.
4. Kutznetsova, N., and S. Leikin. 1999. Does the triple helical domain of type I collagen encode molecular recognition and fiber assembly while telopeptides serve as catalytic domains. *J. Bio. Chem*. **274**:36083-36088.

5. Leikin, S., D. Rau, and V. Parsegian. 1994. Direct measurement of forces between self-assembled proteins: Temperature-dependent exponential forces between collagen triple helices. *Proc. Natl. Acad. Sci*. USA. **91**:276-280.
6. Leikina, E., M. Mertts, N. Kuznetsova, and S. Leikin. 2002. Type I collagen is thermally unstable at body temperature. *Proc. Natl. Acad. Sci.* USA. **99**:1314-1318.
7. O' Shaughnessy, T., H. Lin, and W. Ma. 2003. Functional synapse formation among rat cortical neurons grown on three-dimensional collagen gels. *Neuroscience Letters*. **340**:169 - 172.
8. Park, D., D. Choi, H. Ryu, H. Kwon, H. Joo, and C. Min. 2003. A well-defined in vitro three-dimensional culture of human endometrium and its applicability to endometrial cancer invasion. *Cancer Letters*. **195**:185-192.
9. Ritty, T., and J. Herzog. 2003. Tendon cells produce gelatinases in response to type I collagen attachment. *J. Ortho. Res*. **21**:442-450.
10. Van Oostveldt, K., M. Paape, and C. Burvemich. 2002. Apoptosis of bovine neutrophils following diapedesis through a monolayer of endothelial and mammary epithelial cells. *J. Dairy Sci. Ass.* **85**:139-147.

Related Products:

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
3440-100-01	Cultrex® Rat Collagen I	100 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

Cultrex®

Bovine Collagen I
Catalog #: 3442-050-01
Storage: 4 °C
TREVIGEN®
1-800-873-8443