Cultrex® Bovine Collagen I

Catalog #: 3442-100-01  Size: 20 mL  Concentration: 5 mg/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 \( \alpha_1(II) \) chains and one \( \alpha_2(II) \) 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.

Source: Fetal bovine extensor tendons

Storage Buffer: 20 mM Acetic Acid

Storage Conditions: Product is stable for at least 3 months from the date of receipt when stored at 2 - 8° C. Do not freeze.

Specifications:
- **Gelling**: Type I collagen forms a firm gel at a 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. Collagen I gelling at concentrations below 1 mg/mL may be unforgiving regarding neutralization, and as a result, may require optimization.

1. Place the following on ice:
   - A. Type I Collagen
   - B. Sterile 10X PBS
   - C. Sterile distilled water (dH\(_2\)O)
   - D. Sterile 1 N 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 PBS (phosphate-buffered saline), neutralized by 1 N NaOH.

   A. Volume of collagen needed = \( \frac{(\text{Final conc. of collagen}) \times (\text{Total Volume})}{\text{Initial conc. of collagen}} \)

   B. Volume of 10X PBS needed = \( \frac{\text{Total Volume}}{10} \)

   C. Volume of 1 N 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 1 N NaOH.

5. Add the dH\(_2\)O to the tube and pipet up and down to mix.

6. Add the Collagen I to the tube and pipet up and down to mix.

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 the 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 the 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 the plate or dish, and place the lid containing the sponge on the chamber.

6. Incubate for 5 minutes at 37° C.

7. Remove the 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 \( \mu \)g/cm\(^2\) is recommended.

1. Determine the volume needed for experimentation.

2. Dilute the collagen to 50 \( \mu \)g/mL in 0.02 M acetic acid at the final volume needed.

   A. Volume of collagen = \( \frac{(50 \mu \text{g/mL of collagen}) \times (\text{Final Volume})}{(\text{Initial concentration of collagen})} \)

   B. Volume of 0.02 M acetic acid = Final Volume - Volume of Collagen (Step A)

3. Add solution to plates or dishes at 5 \( \mu \)g/cm\(^2\) (e.g. 50 \( \mu \)g or 1 mL of 50 \( \mu \)g/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 (18 - 24° C) for 1 hour.

5. Carefully aspirate the solution from the well or dish.

6. Rinse the 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:


3. Determine the amount of reagents needed so that collagen I is at the desired concentration in 1X PBS (phosphate-buffered saline), neutralized by 1 N NaOH.

A. Volume of collagen needed = \((\text{Final conc. of collagen}) \times \text{(Total Volume)}\) / \(\text{Initial conc. of collagen}\)

B. Volume of 10X PBS needed = Total Volume / 10

C. Volume of 1 N NaOH needed = (Volume of Collagen) x 0.035 mL

D. Volume of dH\textsubscript{2}O needed = Total Volume - (calculated volumes from steps A+B+C)

4. In a clean sterile tube, mix the 10X PBS and 1 N NaOH.

5. Add the dH\textsubscript{2}O to the tube and pipet up and down to mix.

6. Add the Collagen I to the tube and pipet up and down to mix.

7. Place the collagen into the desired plates or dishes. Solution is stable for 2 - 3 hours on ice.

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Optimization for desired protein concentration may be required. A starting concentration of 5 \(\mu\text{g/cm}^2\) is recommended.

1. Determine the volume needed for experimentation.

2. Dilute the collagen to 50 \(\mu\text{g/mL}\) in 0.02 M acetic acid at the final volume needed.

A. Volume of collagen = \((50 \text{ \(\mu\text{g/mL}\) of collagen}) \times \text{(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 \(\mu\text{g/cm}^2\) (e.g. 50 \(\mu\text{g}\) or 1 mL of 50 \(\mu\text{g/mL}\) of collagen is required for coating a 35 mm dish, which has a surface area of approximately 10 \(\text{cm}^2\)).

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