Collagen, Rat Tail

Catalog Number **5056**

Product Description

Type I collagen is a major structural component of skin, bone, tendon, and other fibrous connective tissues, and differs from other collagens by its low lysine hydroxylation and low carbohydrate composition. Although a number of types of collagen have been identified, all are composed of molecules containing three polypeptide chains arranged in a triple helical conformation. Slight differences in the primary structure (amino acid sequence) establish differences between the types. The amino acid sequence of the primary structure is mainly a repeating motif with glycine in every third position with proline or 4-hydroxyproline frequently preceding the glycine residue.[1,2] Type I collagen is a heterotrimer composed of two α1(I) chains and one α2(I) chain, which spontaneously form a triple helix scaffold at neutral pH and 37°C.

Control of cell growth, differentiation, and apoptosis in multicellular organisms is dependent on adhesion of cells to the extracellular matrix (ECM). Given that Type I collagen is an abundant component of the ECM, cells cultured in three dimensional (3D) collagen gels simulate the *in vivo* cell environment better than traditional 2D systems. This has been shown for a number of cell types including cardiac and corneal fibroblasts, hepatic stellate cells (HSCs), and neuroblastoma cells.[3-6]

Several diseases can affect the mechanical properties of the ECM while other disease states may be caused by changes in the density or rigidity of the ECM. Since Type I collagen is a key determinant of tensile properties of the ECM, 3D collagen gels are useful in studies of mechanotransduction, cell signaling involving the transformation of mechanical signals into biochemical signals.[6-9]

3D gels allow for the study of the effects of the mechanical properties of the ECM, such as density and rigidity, on cell development, migration, and morphology. Unlike 2D systems, 3D environments allow cell extensions to simultaneously interact with integrins on all cell surfaces, resulting in the activation of specific signaling pathways.

Gel stiffness or rigidity also affects cell migration differently in 3D versus 2D environments. Furthermore, integrin independent mechanical interactions resulting from the entanglement of matrix fibrils with cell extensions are possible in 3D systems, but not in 2D systems where the cells are attached to a flat surface.[10-12]

Different collagen subtypes are recognized by a structurally and functionally diverse group of cell surface receptors, which recognize the collagen triple helix. The best-known collagen receptors are the integrins α1β1 and α2β1. α1β1 is the major integrin on smooth muscle cells, while α2β1 is the major form on epithelial cells and platelets. Both forms are expressed on many cell types including fibroblasts, endothelial cells, osteoblasts, chondrocytes, and lymphocytes.[13-15] Some cell types may also express other collagen receptors such as glycoprotein VI (GPVI), which mediates both adhesion and signaling in platelets.[16] Other collagen receptors include discoidin domain receptors, leukocyte-associated IG-like receptor-1, and members of the mannose receptor family.[17,18]

This product is prepared and extracted from rat tail tendon. It is supplied as a sterile 0.2M acetic acid solution with a concentration of 4 mg/ml of collagen. The product is sterilized and has been tested and confirmed negative, for bacterial and fungal contamination.

This collagen product is provided in user-friendly packaging for use and storage. This product is sterile filtered and is supplied as a ready to use solution.

This product is ideal for coating of surfaces, providing preparation of thin layers for culturing cells, or use as a solid gel. Rat Tail Collagen is suitable for applications using a variety of cell lines including hepatocytes, fibroblasts and epithelial cells.

Characterization

Purity: Rat Tail collagen is a pure collagen (>95%) as demonstrated and tested by electrophoresis. SDS PAGE shows the typical α, β and γ banding pattern.

Concentration: The concentration of Rat Tail collagen is approximately 4.0 mg/mL. The actual concentration is printed on the product label and certificate of analysis for each specific lot.

pH: Supplied in 0.2M acetic acid (pH ~3.0).

Sterility: Tested and confirmed negative for bacterial and fungal contamination.

Endotoxin: ≤ 1.0 EU/ml

Storage: This product is stored at 2–10 °C and is shipped on frozen gel packs.

Stability: The product shelf life is 12 months when storage at 2–10 °C. Expiration date is listed on the packaging label.

Cell Adherence Assay: To demonstrate the bioactivity, human dermal fibroblasts were seeded onto surfaces coated with Rat Tail collagen in serum free conditions. All surfaces were blocked with a solution containing 1% BSA. Cells were then allowed to attach for one (1) hour at 37°C. The results indicate significant cell attachment bioactivity of Rat Tail collagen. The control surfaces showed only minimal cell adherence.

Precautions and Disclaimer
This product is for R&D use only and is not intended for human or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Coating Procedure

Note: Employ aseptic practices to maintain the sterility of the product throughout the preparation and handling of the collagen and other solutions.

1. Transfer desired volume of collagen solution from the bottle to a dilution vessel if required. Further dilute to desired concentration using sterile 0.1% acetic acid solution. A typical working concentration may range from 10 to 100 µg/ml.

Note: Use these recommendations as guidelines to determine the optimal coating conditions for your culture system.

2. Add appropriate amount of diluted Rat Tail collagen to the culture surface.

3. Incubate at room temperature or 37°C, covered, for 1-2 hours.

4. After incubation, aspirate any remaining material.

5. Rinse coated surfaces carefully with sterile medium or PBS, avoid scratching surfaces.

6. Coated surfaces are ready for use. They may also be stored at 2-8°C damp or air dried if sterility is maintained.

3-D Gel Preparation Procedure

Note: It is recommended that the collagen and other working solutions be chilled and kept on ice during the preparation of the collagen.

1. Place the following reagents on ice:
   - Type I Rat Tail collagen (4 mg/ml)
   - Sterile PBS (10X)
   - Sterile Cell Culture Water (dH_2O)
   - Sterile 1 N NaOH

2. Determine the final volume and concentration required of Rat Tail collagen required.

3. Determine the amounts of reagents required to yield Rat Tail collagen at the concentration and pH required:

a. Volume of Collagen needed = $\frac{\text{(Final Concentration) X (Total Volume)}}{\text{(Initial Concentration of Collagen)}}$

b. Volume of 10X PBS needed = $\frac{\text{Total Volume}}{10}$

c. Volume of 1N NaOH needed = Volume of Collagen X 0.017 ml

d. Volume of dH_2O needed = Total Volume – (Sum of a + b+ c)

4. Mix the volumes calculated for 10X PBS, NaOH and dH2O in a sterile tube.

5. Add Rat Tail collagen to the tube with the reagents and pipet up and down to mix. Vortexing is not recommended.

6. Dispense the Rat Tail collagen mixture in the desired sterile plates or culture vessels.

7. Incubate at 37°C for 1 hour for gel formation.

References

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