Directions for Use
TeloCol®- 10

PURIFIED TYPE I BOVINE TELO-COLLAGEN SOLUTION, 10 MG/ML

Catalog Number **5226**

Product Description

Advanced BioMatrix offers TeloCol®-6 collagen solution which is highly purified telo-collagen at approximately 6 mg/mL, pH 2, and is sterile filtered. TeloCol® is about 97% Type I collagen with the remainder being comprised of Type III collagen. The purity of the TeloCol® collagen is ≥99%. SDS-PAGE electrophoresis shows the typical α, β and γ banding pattern for collagen. The actual collagen concentration is printed on the product label and certificate of analysis for each specific lot.

TeloCol®-6 is derived from an acid extraction process yielding a telopeptide-intact collagen. The pro-peptide regions at both ends of the collagen chain, N- and C-telopeptide regions, are maintained.

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 mechano-transduction, 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 from collagen extracted from bovine hide and contains a high monomer content. Starting material was isolated from a closed herd and purified using a manufacturing process following applicable aspects of cGMP. This process contains built-in, validated steps to insure inactivation of possible prion and/or viral contaminants.

Characterization

Parameter/Test/Method	Specification
Collagen Concentration (mg/ml) - Biuret	9.0 – 11.0
Purity - SDS PAGE Electrophoresis – Silver staining	≥ 99%
Electrophoretic Pattern - SDS PAGE Electrophoresis - Coomassie	≥ 85% collagen contained with α, β and γ, < 15% collagen contained within bands traveling faster than alpha
pH	1.9 – 3.0
Osmolality (mOsmo H2O/Kg)	≤ 35
Gel Formation Tube Test (minutes)	≤ 40
Kinetic Gel Test (minutes)	≤ 40
Fibrillogenesis (Absorbance Units)	≥ 0.35
Sterility (USP modified)	No Growth
Endotoxin LAL (EU/ml)	≤ 10
Gel Stiffness Plateau	Characteristic
Cell Attachment	Pass

Storage/Stability: The product is stored at 2–10°C and ships on frozen gel packs. Do not freeze. The expiration date is listed on the product label and certificate of analysis for each specific lot. The expiration date is applicable when product is handled and stored as directed.

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 product.

1. Transfer desired volume of TeloCol®-10 collagen solution from the bottle to a dilution vessel if required. Further dilute to desired concentration using a sterile 0.01 N HCl solution. A typical working concentration may range from 50 to 100 µg/ml.

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

2. Swirl contents gently until material is completely mixed.

3. Add appropriate amount of diluted TeloCol®-10 material to the culture surface ensuring that the entire surface is coated.

4. Incubate at room temperature, covered, for 1-2 hours. Aspirate any remaining material. Alternatively, incubate at room temperature until surface is dry.

5. After incubation, aspirate any remaining material.

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

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

Note: It is recommended that the collagen and other working solutions be chilled and kept on ice during the preparation of the collagen. This product will polymerize very quickly.

Note: Due to the high concentration and viscosity of the collagen, using pipettes for mixing and weighing will lead to high yield loss.

1. Determine the desired volume of collagen required.

2. Slowly transfer 1 part of chilled 10X PBS or 10X culture media to 8 parts chilled collagen. Mix via gentle mechanical stirring.

3. Adjust pH of mixture to 7.0-7.5 using sterile 0.1M NaOH (~ 1 part but monitor the pH). Monitor pH adjustment with pH paper or Phenol Red). Mix via gentle mechanical stirring. **Vortexing is not recommended.**

4. Dispense the collagen mixture in the desired sterile plates or culture vessels.

5. Incubate at 37°C for 1 hours for full gel formation.

References

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