ADVANCED BIOMATRIX
A BICO COMPANY

Directions for Use
VitroCol®

PURIFIED HUMAN TYPE I ATELO-COLLAGEN SOLUTION, 3 MG/ML

Catalog Number **5007**

Product Description

Advanced BioMatrix offers VitroCol® collagen solution which is highly purified atelo-collagen at approximately 3 mg/mL, pH 2, and is sterile filtered. VitroCol® is about 97% Type I collagen with the remainder being comprised of Type III collagen. The purity of the VitroCol® 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.

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 $\alpha_1\beta_1$ and $\alpha_2\beta_1$. $\alpha_1\beta_1$ is the major integrin on smooth muscle cells, while $\alpha_2\beta_1$ 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 extracellular matrix secreted by normal human fibroblasts and contains a high monomer content. The fibroblasts cells used as the starting materials were intensively tested. The fibroblasts were grown in a bioreactor and secreted extracellular matrix materials. This extracellular matrix material was purified using a multi-step manufacturing process with applicable aspects of cGMP. This process contains built-in, validated steps to insure inactivation of possible prion and/or viral contaminants.

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Characterization and Testing

Parameter/Test/Method	Specification
Collagen Concentration (mg/ml) - Biuret	2.9 – 3.2
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 – 2.1
Osmolality (mOsmo H2O/Kg)	≤ 35
Gel Formation Tube Test (minutes)	≤ 40
Kinetic Gel Test (minutes)	≤ 40
Fibrillogenesis (Absorbance Units)	> 0.5
Sterility (USP modified)	No Growth
Endotoxin LAL (EU/ml)	≤ 5.0
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: Use these recommendations as guidelines to determine the optimal coating conditions for your culture system.

1. Remove required quantity of collagen from the bottle and dispense into a dilution vessel.

2. Dilute VitroCol® in water to ~50 to 100 µg/ml (~1:30). A 0.01 N HCl solution may also be used.

3. Swirl contents gently until material is completely mixed.

4. Add appropriate amount of diluted VitroCol® material to the culture surface ensuring that the entire surface is coated.

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

6. After incubation, aspirate any remaining material.

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

8. 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

1. Slowly add 1 part of chilled 10X PBS or 10X culture media to 8 parts of chilled collagen solution with gentle swirling.

2. Adjust pH of mixture to 7.0–7.5 using sterile 0.1 M NaOH. Monitor pH adjustment carefully (pH meter, phenol red, or pH paper).

3. Adjust final volume to a total of 10 parts with sterile water.

4. To prevent gelation, maintain temperature of mixture at 2–10° C.

5. To form gel, incubate at 37°C in humidified incubator for 60 minutes or until a firm gel is formed.

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