

VitroCol®
PURIFIED HUMAN COLLAGEN, TYPE I, LYOPHILIZED, STERILIZED
Catalog Number **5008**

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 $\alpha 1(I)$ chains and one $\alpha 2(I)$ 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

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. It is supplied as a sterile lyophilized powder with 15 mg of collagen. The human fibroblast cells used to produce this product were intensively tested. Production and purification of the collagen occurs using a

multi-step manufacturing process. This process contains built-in, validated steps to insure inactivation of possible viral contaminants. The product is sterilized and has been tested and confirmed negative for bacterial and fungal contamination.

Advanced BioMatrix's Type I, human collagen from fibroblast cell culture is provided as a lyophilized powder in a 15 mg sterile package size. When reconstituted with 5 ml of 0.01 N HCl, the resulting concentration is 3 mg/ml.

Note: This product is intended for the coating of surfaces including plasticware but is not intended for use as a 3D gel.

Characterization

Purity: VitroCol® is ultrapure collagen (~99.9% SDS-PAGE, ~97% Type I with remainder Type III collagen). SDS-PAGE shows the typical α , β and γ banding pattern.

Quantity: Each vial contains 15 mg of collagen. To achieve a 3 mg/ml concentration, reconstitute with 5 ml of sterile 0.01 N HCl. This will result in a solution with a pH ~2.

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

Endotoxin: \leq 1.0 EU/ml

Storage: This product is stored at -20°C prior to solubilization and is shipped on frozen gel packs. The product is recommended to be stored at 2–10 °C after reconstitution.

Stability: The product shelf life is 24 months when storage at -20°C. The product shelf life after reconstitution is 3 months.

Cell Adherence Assay: To demonstrate the bioactivity, human dermal fibroblasts were seeded onto surfaces coated with VitroCol® 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 VitroCol®. 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

1. Add 5 ml of sterile 0.01 N HCl solution to the VitroCol[®] serum vial containing 15 mg.
 2. Gently mix contents vigorously until material is completely solubilized.
 3. Transfer desired volume of solution from the serum vial to a dilution vessel if required. Further dilute to desired concentration using sterile 0.01 N HCl 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.
4. Add appropriate amount of diluted VitroCol[®] material to the culture surface.
 5. Incubate at room temperature or 37°C, covered, for 1-2 hours.
 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 2-10°C damp or air dried if sterility is maintained.

References

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