

Directions for Use TeloCol® - 6

PURIFIED TYPE I BOVINE TELO-COLLAGEN SOLUTION, 6 MG/ML
Catalog Number 5225

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 $\alpha 1(I)$ 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 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 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	5.8 – 6.8
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 H ₂ O/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®-6 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®-6 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 using the supplied Neutralization Solution

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.

1. Determine the desired volume of collagen required.

2. Transfer 1 part of chilled neutralization solution into a sterile mixing vessel or tube.

3. Transfer 9 parts of chilled TeloCol®-6 collagen solution into the sterile mixing vessel or tube for a total of 10 parts.

4. Gently agitate the mixture or pipet up and down to mix. Vortexing is not recommended.

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

6. Incubate at 37°C for 1 hours for gel formation.

3-D Gel Preparation Procedure without using the supplied Neutralization Solution

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

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). Mix with gentle swirling.

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

4. To prevent gelation, maintain temperature of mixture at 2–10°C. Dispense the *TeloCol*[®]-6 collagen mixture in the desired sterile plates or culture vessels.

5. To form gel, warm to 37°C. Allow approximately 30 to 60 minutes for gel formation.

collagen receptor different from another? *Conn. Tiss.*, **30**, 273-283 (1998).

16. Clemetson, K.J., and Clemetson, J.M., Platelet collagen receptors. *Thromb Haemost.*, **86**, 189-197 (2001).

17. Leitinger, B., and Hohenester, E., Mammalian Collagen Receptors, *Matrix Biol.*, **26**, 146-155 (2007).

18. Popova, S.N., et al., Physiology and pathology of collagen receptors. *Acta Physiol. (Oxf)*, **190**, 179-187 (2007).

References

1. Tanzer, M. L., Cross-linking of collagen. *Science*, **180**(86), 561-566 (1973).
2. Bornstein, P., and Sage, H., Structurally distinct collagen types. *Ann. Rev. Biochem.*, **49**, 957-1003 (1980).
3. Tomasek, J.J., and Hay, E.D., Analysis of the role of microfilaments in acquisition and bipolarity and elongation of fibroblasts in hydrated collagen gels. *J. Cell Biol.*, **99**, 536-549 (1984).
4. Karamichos, D., et al., Regulation of corneal fibroblast morphology and collagen reorganization by extracellular matrix mechanical properties. *Invest. Ophthalmol. Vis. Sci.*, **48**, 5030-5037 (2007).
5. Sato, M., et al., 3-D Structure of extracellular matrix regulates gene expression in cultured hepatic stellate cells to induce process elongation. *Comp Hepatol.*, Jan 14;3 Suppl 1:S4 (2004).
6. Li, G.N., et al., Genomic and morphological changes in neuroblastoma cells in response to three-dimensional matrices. *Tissue Eng.*, **13**, 1035-1047 (2007).
7. Roeder, B.A., et al., Tensile mechanical properties of three-dimensional type I collagen extracellular matrices with varied microstructure. *J. Biomech. Eng.*, **124**, 214-222 (2002).
8. Wozniak, M.A., and Keely, P.J., Use of threedimensional collagen gels to study mechanotransduction in T47D breast epithelial cells. *Biol. Proced. Online*, **7**, 144-161 (2005).
9. Grinnell, F., Fibroblast biology in three-dimensional collagen matrices. *Trends Cell Biol.*, **13**, 264-269 (2003).
10. Beningo, K.A., et al., Responses of fibroblasts to anchorage of dorsal extracellular matrix receptors. *Proc. Natl. Acad. Sci. USA*, **101**, 18024-18029 (2004).
11. Zaman, M.H., et al., Migration of tumor cells in 3D matrices is governed by matrix stiffness along with cell-matrix adhesion and proteolysis. *Proc. Natl. Acad. Sci. USA*, **103**, 10889-10894 (2006).
12. Jiang, H., and Grinnell, F., Cell-matrix entanglement and mechanical anchorage of fibroblasts in three-dimensional collagen matrices. *Mol. Biol. Cell*, **16**, 5070-5076 (2005).
13. Heino, J., The collagen receptor integrins have distinct ligand recognition and signaling functions. *Matrix Biol.*, **19**, 319-323 (2000).
14. Heino, J., The collagen family members as cell adhesion proteins. *BioEssays*, **29**, 1001-1010 (2007).
15. Ivaska, J., et al., Cell adhesion to collagen-is one