Collagen Solution, 3 mg/ml from human fibroblast

Catalog Number C2249
Storage Temperature 2–8 °C

Product Description
Type I collagen is a major structural component of skin, bone, tendon, and other fibrous connective tissues. It differs from other collagens by its low lysine hydroxylation and low carbohydrate composition. A number of types of collagen have been identified and 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 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 Type I collagen is an abundant component of the ECM, cells cultured in three dimensional (3D) collagen gels simulate the \emph{in vivo} cell physiology better than traditional 2D systems. This has been shown for a number of cell types including cardiac and corneal fibroblasts, hepatic stellate cells (HSC), and neuroblastoma cells.\(^3,6\)

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 utilize integrins on both the dorsal and ventral 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.\(^7,8\)

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 the 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.\(^6,10-12\)

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 integrin \(\alpha_1\beta_1\) and \(\alpha_2\beta_1\). Integrin \(\alpha_1\beta_1\) is the major form on smooth muscle cells, while \(\alpha_2\beta_1\) is the major integrin 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 the extracellular matrix secreted by normal human fibroblasts and contains a high monomer content. \emph{In vitro} cultures were prepared using intensively tested human fibroblast cells 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.

It is supplied as a \(\sim\)3 mg/ml (0.3%) aqueous solution in 0.01 M HCl (pH \(\sim\)2.0). The product is sterilized by membrane filtration and has been tested, and confirmed negative, for bacterial and fungal contamination.

Endotoxin: <0.5 EU/ml (LAL assay)
Purity: ≥99.9% (SDS-PAGE)

(∼97% Type I with remainder Type III collagen)
SDS-PAGE shows the typical band pattern.
Gradual breakdown may occur over long periods of
time thus creating atypical banding patterns.

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

Storage/Stability
The product ships on wet ice and storage at 2–8 °C is
recommended. Do not freeze. Expiration date is on
label.

Procedure
3-D Gel Preparation
1. Mix 8 parts of chilled collagen solution with 1 part of
10× PBS (Catalog Number P5493 or P5368) or
10× culture medium. Cells may be added following
this step.
2. Adjust pH of mixture prepared in step 1 to 7.2–7.6.
Use of 0.1 M NaOH (10-fold dilution of Catalog
Number S2770) or 0.01 M HCl (100-fold dilution of
Catalog Number H9892) is recommended. Monitor
pH adjustment carefully with pH meter, phenol red,
or pH paper.
3. To prevent gelation, maintain temperature of the
mixture at 2–8 °C. To form gel, warm to 37 °C. For
best results allow 45 minutes to 1 hour for gel
formation.
4. The gels can be dried under a laminar flow hood.

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