

## Directions for Use RatCol®

PURIFIED RAT TAIL TYPE I TELO-COLLAGEN SOLUTION, 100 MG  
WITH NEUTRALIZATION SOLUTION

Catalog Number: **5153**

### Product Description

Advanced BioMatrix offers RatCol® collagen solution which is highly purified telo-collagen (100 mg) at approximately 4 mg/mL, pH 3-4, and is sterile filtered. RatCol® is about 97% Type I collagen with the remainder being comprised of Type III collagen. The purity of the RatCol® collagen is ≥99%. SDS-PAGE electrophoresis shows the typical  $\alpha$ ,  $\beta$  and  $\gamma$  banding pattern for collagen. The actual collagen concentration is printed on the product label and certificate of analysis for each specific lot. The collagen is accompanied with a bottle of pre-formulated neutralization solution for the formation of a collagen gel.

RatCol® is derived from an acid extraction process yielding a telopeptide-intact collagen in 0.02M acetic acid buffer. 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.<sup>1,2</sup> 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.<sup>3-6</sup>

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.<sup>6-9</sup> 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.<sup>10-12</sup>

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.<sup>13-15</sup> Some cell types may also express other collagen receptors such as glycoprotein VI (GPVI), which mediates both adhesion and signaling in platelets.<sup>16</sup> Other collagen receptors include discoidin domain receptors, leukocyte-associated IG-like receptor-1, and members of the mannose receptor family.<sup>17,18</sup>

This product is prepared from collagen extracted from rat tail tendons and contains a high monomer content. Starting material was isolated from Sprague Dawley rats from a managed colony 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. This product is ideal for coating of surfaces, providing preparation of thin layers for culturing cells, or use as a solid gel.

## Characterization

Parameter/Test/Method	Specification
Collagen Concentration (mg/ml) - Biuret	3.5 – 4.5
Purity - SDS PAGE Electrophoresis – Silver staining	≥ 99%
Electrophoretic Pattern - SDS PAGE Electrophoresis - Coomassie	≥ 85% collagen contained with $\alpha$ , $\beta$ and $\gamma$ , < 15% collagen contained within bands traveling faster than alpha
pH	3.0 – 3.8
Osmolality (mOsmo H <sub>2</sub> O/Kg)	≤ 35
Gel Formation Tube Test (minutes)	≤ 40
Kinetic Gel Test (minutes)	≤ 40
Sterility (USP modified)	No Growth
Endotoxin LAL (EU/ml)	≤ 10.0
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: Employ aseptic practices to maintain the sterility of the product throughout the preparation and handling of the collagen and other solutions.*

1. Transfer desired volume of collagen solution from the bottle to a dilution vessel if required. Further dilute to desired concentration using sterile 0.1% acetic acid 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. Add appropriate amount of diluted Rat Tail collagen to the culture surface.

3. Incubate at room temperature, covered, for 1-2 hours. Aspirate any remaining material. Alternatively, incubate at

room temperature until surface is dry.

4. After incubation, aspirate any remaining material.

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

6. 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 the chilled neutralization solution into a sterile mixing vessel or tube.

3. Transfer 9 parts of the Rat Tail Collagen 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 Rat Tail Collagen mixture into the desired plates or culture vessels.

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

## References

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