

## Directions for Use

RatCol® High Concentration Type I Acid Soluble Rat Tail Collagen contains 100 mg at a concentration of approximately 10 mg/mL in a 0.02M acetic acid solution (~pH 3). RatCol® collagen is soluble *telo*-collagen. This collagen product is provided in user-friendly packaging for use and storage. This product is sterile filtered and is supplied as a ready to use solution.

Note: the 10 mg/ml collagen is highly viscous. Ensure thorough mixing for product to polymerize properly.

## 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 ug/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. Rinse coated surfaces carefully with sterile medium or PBS, avoid scratching surfaces.
- 5. 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

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. 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  $2-10^{\circ}$  C.
- 5. To form gel, warm to 37° C. Allow approximately 90 to 120 minutes for gel formation.