

**COLLAGEN, TYPE IV, HUMAN,  
LYOPHILIZED, STERILIZED**  
Catalog Number **5022**

### Product Description

Type IV collagen is the primary collagen found in the extracellular basement membranes separating a variety of epithelial and endothelial cells. It is a major component of the dermal-epidermal junction where it is mostly found in the lamina densa. It is a heterotrimeric molecule containing two  $\alpha 1$ -like and one  $\alpha 2$ -like chains.

This Type IV collagen is isolated from human placenta and is purified using a multi-step process. The product is supplied as a sterile, lyophilized powder containing 5 mg of Type IV collagen per vial.

Type IV human collagen is typically used as a thin coating on tissue culture surfaces. This product is generally used *in vitro* as a substrate scaffold to enhance cell attachment, adherence and proliferation. Type IV collagen may be used to culture epithelial, endothelial, muscle, nerve and many other cell types. Additionally, this product is suitable for use as a substrate for collagenase assays and positive controls.

### Characterization

**Identity/Purity:** The identity and purity of Type IV Human Collagen is qualitatively evaluated using electrophoresis (SDS-PAGE) which shows the typical banding pattern for Type IV collagen.

**Storage:** This product is stored at  $-20^{\circ}\text{C}$  prior to solubilization and is shipped on frozen gel packs. If entire amount of material is not used immediately, dispense into appropriate aliquots and store frozen. Avoid repeated freeze and thaw.

**Stability:** The product shelf life is 36 months when stored at  $-20^{\circ}\text{C}$ . The product shelf life after reconstitution is 3 months when stored at  $2-10^{\circ}\text{C}$ .

**Cell Adherence Assay:** To demonstrate the bioactivity, human dermal fibroblasts were seeded onto surfaces coated with Type IV Collagen 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  $37^{\circ}\text{C}$ . The results indicate significant cell attachment bioactivity of Type IV Collagen. The control surfaces showed minimal and poor 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.

The raw material source for this product is human placenta. The raw material has been tested for the presence of infectious viruses (HIV 1 & 2, HBV, HCV) and found nonreactive. However, no known test method can offer complete assurance of safety. Appropriate safety and personal protective practices should be followed when handling this product.

### Preparation Procedure

1. Reconstitute the 5 mg vial with 5 ml of cold, sterile 0.25% acetic acid (*not 25 mM*). Gently mix the vial back and forth until the collagen is fully wetted. Mix at  $2$  to  $8^{\circ}\text{C}$  overnight on a shaker table, slowly swirling the solution.

Note: The resulting solution will be slightly hazy. If there are some insoluble materials present and you wish to remove it, aseptically centrifuge the material for approximately 2 minutes at 3000 RPM.

2. Dilute the product to desired concentration with sterile 0.25% acetic acid. A typical final coating concentration may be  $10$  to  $100\ \mu\text{g}/\text{cm}^2$ . Testing will likely be required to determine optimal concentrations required for different cell culture systems.

3. Add appropriate amount of diluted product to culture surface.

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 excess remaining material.

6. Rinse coated surface carefully with a sterile balanced salt solution. Avoid scratching surfaces.

7. Aspirate remaining material from coated surface.

8. Coated culture vessels are now ready to use. The coated culture vessels may be stored at  $2$  to  $10^{\circ}\text{C}$ .