# WHITE PAPER: FIBRILLAR COLLAGEN FOR RESEARCH

## 1.0 GENERAL INFORMATION

**Material Name:** 65 mg/mL Fibrillar Collagen Type I, in phosphate saline buffer

**Product Number:** FS24001 (research-grade)
Note: Contact info@collagensolutions.com if you wish to purchase medical-grade formulation

**Product Description/ Appearance:** White to off-white, no phase separation, no gaps, no cracks. 1mL in a 3mL syringe.

**Product Analysis:**

| Test / Requirement | Specification |
|---|---|
| **Collagen Concentration** | 60-70 mg/mL (6.0% – 7.0%) |
| **pH** | 7.0 – 7.6 |
| **Appearance** | White to off-white, no phase separation, no gaps or cracks |
| **Electrophoresis Pattern (purity by SDS PAGE)** | ≥ 90% within α, β and γ<br>≤ 10% faster than α |
| **Endotoxin** | <10 EU/mL |
| **Extrusion** | Does not occlude through a 21G needle |

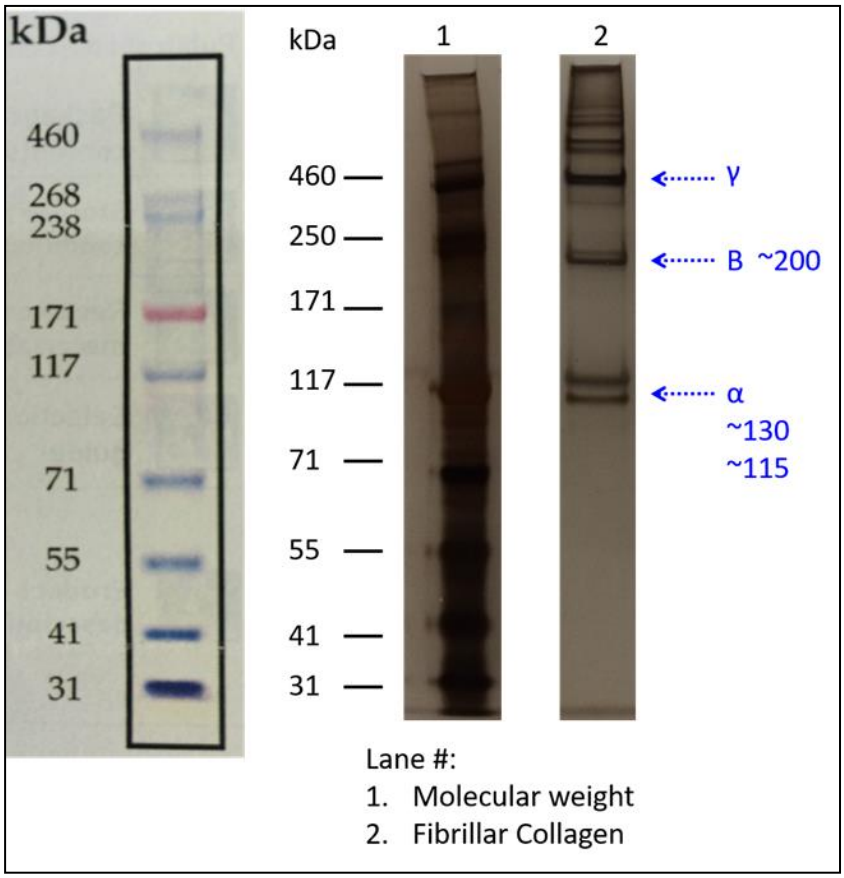


**Figure 1: SDS page and Silver staining showing the regular Collagen type I bands.**

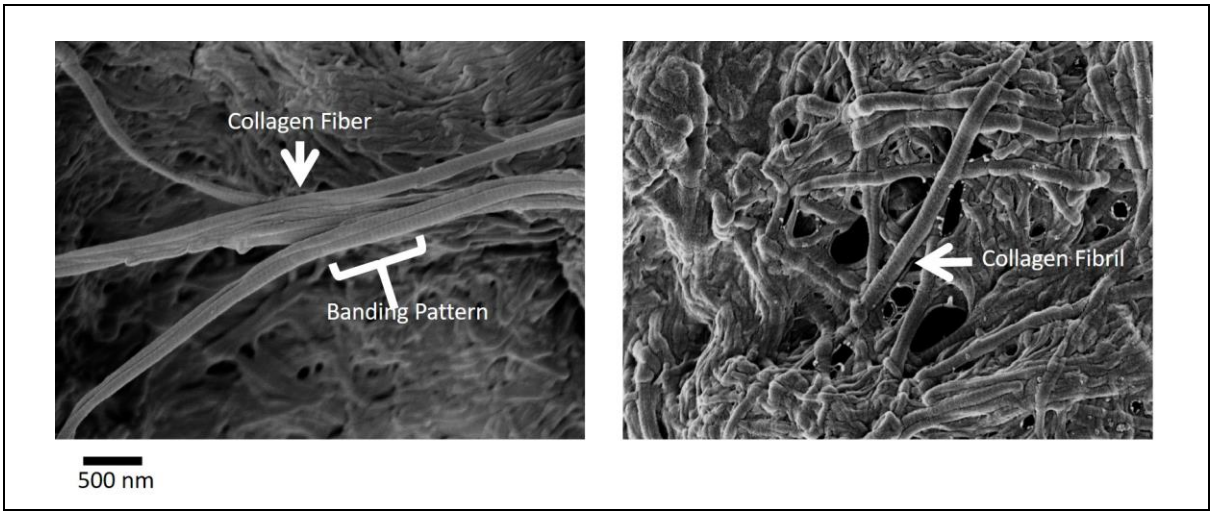


**Figure 2: SEM image of the FS24001.**

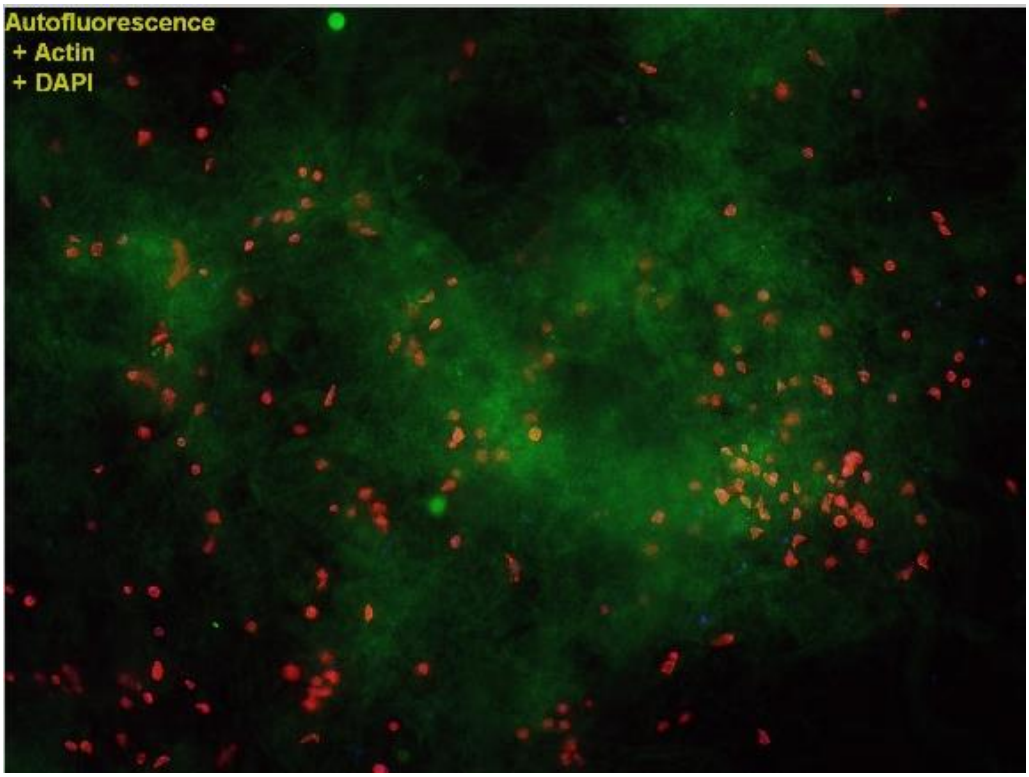


**Figure 3: Keratinocytes sprayed in fibrillar collagen.**

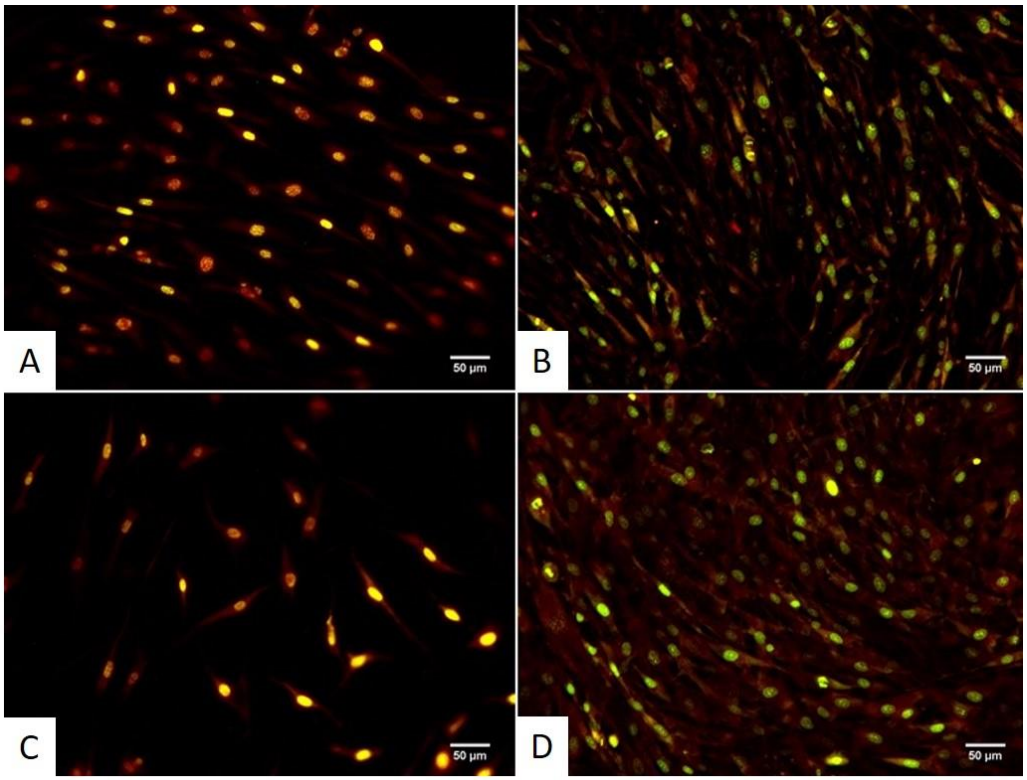


**Figure 4. Fluorescence images of human dermal fibroblasts pipetted or sprayed in collagen and in media stained with PicoGreen (yellow) and phalloidin (red). A) Collagen pipette. B) Media Pipette. C) Collagen spray. D) Media Spray.**

## 2.0 STORAGE REQUIREMENTS

### Storage Conditions

Store at 2 - 8°C

## 3.0 FIBRILLAR COLLAGEN INSTRUCTIONS FOR USE

### Reducing Concentration for Cell or Drug Delivery

1. Mix the desired amount of cell and media to the collagen (the volume of media will dictate the final concentration)
2. To do so, put the cells and media into a 3mL syringe with Luer lock and connect to the collagen syringe with a Femal Luer coupler.
3. Mix syringe-to-syringe approximately 40 times, back and forth (or until the media and collagen are well-mixed)

4. Plate and incubate at 37°C for your experiment (Note: the construct will float when overlaid with media, particularly at lower concentrations)

**3D Gel Protocol (for 10 mg/mL Fibrillar Collagen)**

Required Reagents:

- 65 mg/mL Fibrillar collagen
- 0.01N HCl
- 0.1N HCl
- Neutralizing buffer (for example, 0.2M sodium phosphate, pH 11.2)
- Media and cells (optional, for dilution)

*To prevent premature gelling, ensure that all reagents are chilled before use and kept on ice while experiment is being performed.*

Procedure to create 4mL of 10mg/mL gel:

1. Weigh out 620mg fibrillar collagen in a syringe. Set aside.
2. To a new syringe, combine:
    - 2.2mL 0.01N HCl
    - 400µL 0.1N HCl
3. Solubilize collagen by connecting syringes from steps (1) and (2) and mixing thoroughly. Solution should be between pH 2.0 – 3.0 and will be thick and transparent. Set aside.
4. To a new syringe, add 800µL neutralizing buffer (will be a 1:9 ratio of buffer to collagen).
    - If diluting, also add in desired amount of media and cells. (For example, add 4mL of media and cells to make 8mL of 5mg/mL gel.)
5. Connect syringes from (3) and (4) and mix thoroughly. pH should be approximately 7.0.
6. Spray or pipette into culture plates and incubate undisturbed. Solution should solidify within 45 minutes.
7. Overlay with media if needed and incubate.

## 4.0 REFERENCE

Simpliciano C, Hollenstein J, Burgin J. Collagen as a biological glue for cell spraying in wound healing. Tissue Engineering & Regenerative Medicine Society, Americas Chapter; 2016 Dec 11-14; San Diego, CA.