WHITE PAPER: ATELO COLLAGEN

1.0 GENERAL INFORMATION

Material Name: Pepsin Soluble Atelo Collagen in 0.01M HCl

Product Number: FS22001, FS22002, FS22003, FS22004, FS22005, FS22006

Product Description/ Appearance: Clear, colorless, slightly viscous solution with no particulates or precipitate

Product Analysis:

Test / Requirement	Specification
pH	1.8 – 2.2
Electrophoretic Pattern (purity by SDS PAGE)	≥95% within α, β and γ ≤5% faster than α
Endotoxin	<0.5 EU/mL
Heavy Metals	≤20ppm
Viscosity	22 - 32 mPas*
Sterility	No Growth
Fibrillogenesis	>0.75 Absorbance Units

*1mPas = 1cP

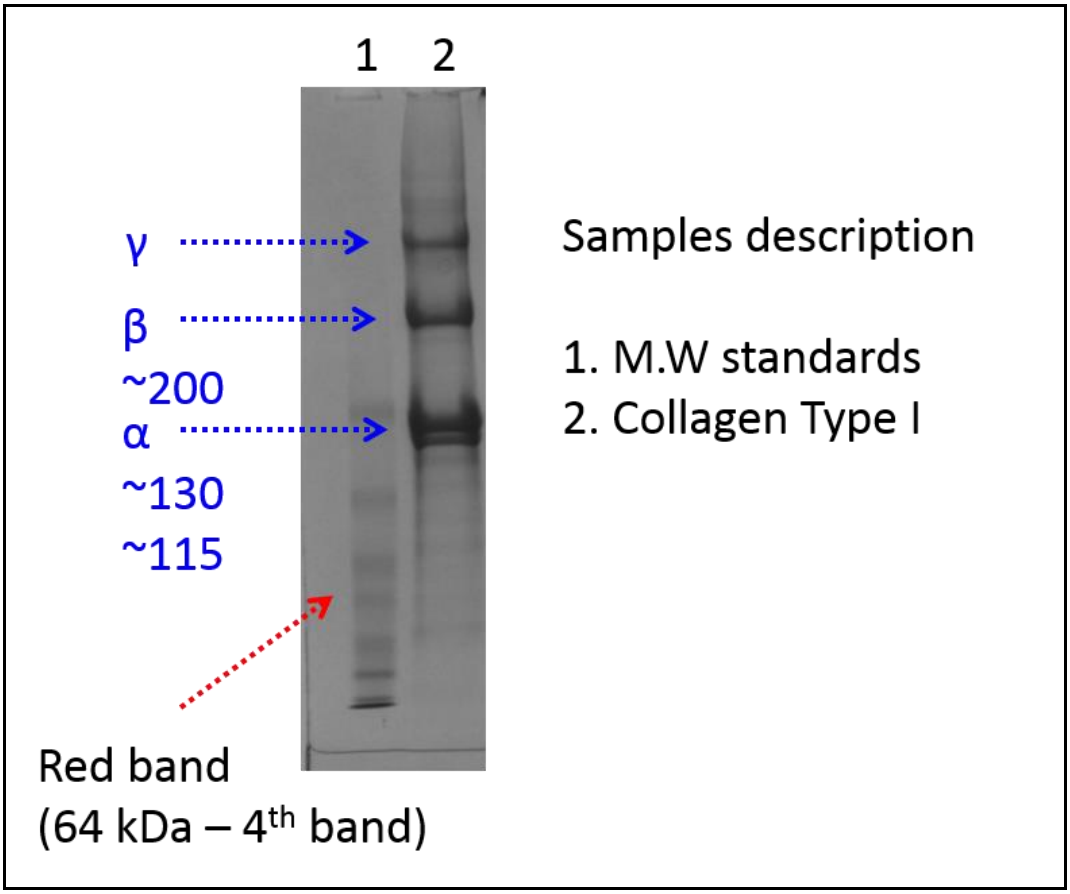

Figure 1: SDS page and Coomassie Blue staining showing the regular Collagen type I bands.

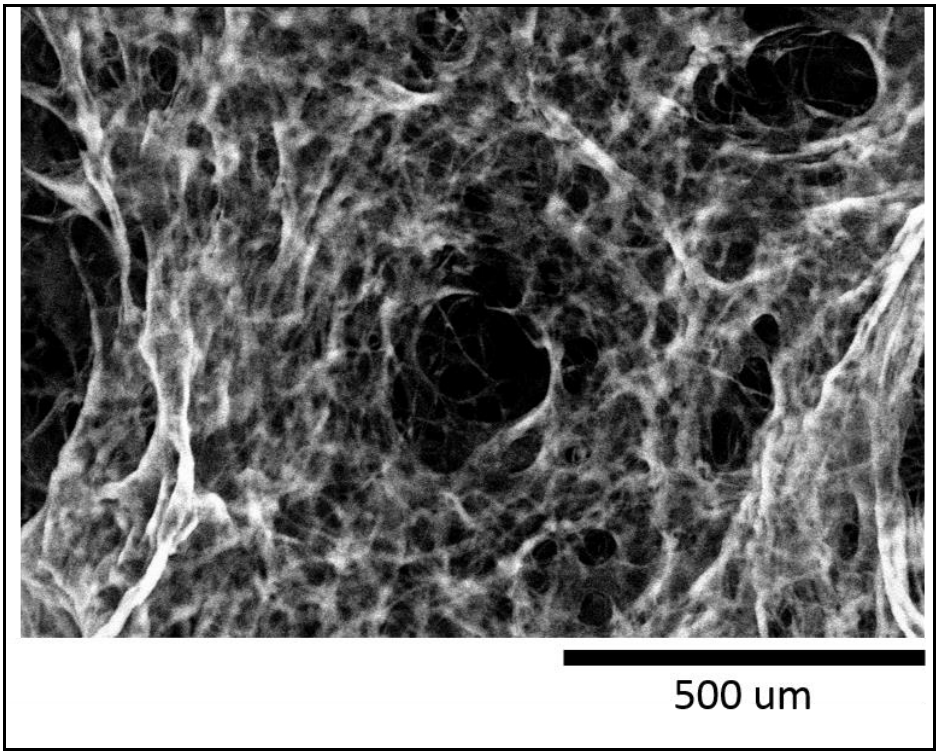

Figure 2: SEM image of the FS22001.

2.0 STORAGE REQUIREMENTS

Storage Conditions
Store at 2 - 8°C

3.0 SOLUBLE COLLAGEN INSTRUCTIONS FOR USE

Three Dimensional Gel using the Titration Buffer

1. Cool the pH 2 collagen solution to 2-10°C
2. Sterile filter the neutralization buffer before use
3. Add 1 part buffer to 9 parts collagen solution and mix (keep cool) i.e. 1 ml neutralization buffer to 9 ml collagen
4. Load into the plasticware cold and then incubate the neutralized collagen at 37°C for at least 45 minutes (do not disturb the gel during gellation as this will cause a weaker gel)

Before using the buffer on a large scale consider checking the pH on a small scale first, i.e., 0.2 ml buffer to 1.8 ml collagen. The desired final pH is 7.0-7.6.

Storage of the buffer: room temperature (note: if the buffer is refrigerated crystals may form. Raise the temperature and mix before use if crystals are present.)

Thin Coating

1. Dilute material to 50-100 µg/ml using 0.01M HCl.
2. Add enough diluted material to coat dishes with 5-10 µg/cm2.
3. Use one to two milliliters for a 35mm dish
4. Incubate at room temperature for one hour
5. Carefully aspirate remaining solution
6. Rinse well to remove acid, using PBS or serum free medium.
7. Plates may be used immediately or air dried. They may then be stored at 2-10 ^{o}C for up to one week under sterile conditions

Ammonia Gel Procedure

1. Prepare ammonia vapor chamber by adding a sterile gauze sponge to the inside lid of petri dish saturate the gauze with ammonium hydroxide. Place lid on the dish and set aside.
2. Using aseptic technique, add soluble collagen to sterile glass or polystyrene culture dishes

Dish size	Soluble collagen amount
100 mm	1 ml
60 mm	0.5 ml
35 mm	0.2 ml

3. Expose collagen coated dishes to ammonia vapor for 2-5 minutes, and then remove collagen dishes from chamber
4. Rinse dishes (with media) three times to remove the ammonium hydroxide
5. Dishes are now ready for use

4.0 REFERENCES

The following reference are examples of different usages of soluble collagen

Agu RU., *et al*. In-vitro Nasal Drug Delivery Studies: Comparison of Derivatised, Fibrillar and Polymerized Collagen Matrix-based human Nasal Primary Culture Systems for Nasal Drug Delivery Studies. *J Pharm Pharmacol*. 2001. 53(11):1447-56

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Evans GR., *et al*. Bioactive Poly(L-lactic acid) Conduits Seeded with Schwann Cells for Peripheral Nerve Regeneration. *Biomaterials*. 2002. 23(3):841-8

Frenkel SR., *et al.* Chondrocyte Transplantation using a Collagen Bilayer Matrix for Cartilage Repair. *J Bone Joint Surg Br.* 1997. 79(5):831-6

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Schwab IR. Cultured Corneal Epitelia for Ocular Surface Disease. *Trans Am Ophthalmol Soc.* 1999. 97:891-986

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