



**Seallagen**

## Directions for Use Marine Essence Biosciences Collagen Solutions

SEALLAGEN AND SEALLAGEN-MCT (LYOPHILIZED AND SOLUTION-3MG/ML)

### Product Description

Marine Essence Biosciences offers collagen solution which is highly purified echinoderm Type-I collagen at approximately 3 mg/mL and is provided gamma sterilized. Seallagen and Seallagen-MCT is >98% and >95%, respectively, Type-I collagen based on SDS-PAGE showing the typical 2  $\alpha$ , 1  $\beta$ , and 1  $\gamma$  banding pattern for collagen.

This product is prepared from collagen extracted from echinoderm mutable collagenous tissue. Starting material was isolated 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.

### Characterization and Testing\*

Parameter	Specification	Method
Color	White to Off White/ Clear to Slightly Opaque	Organoleptic
Appearance	Fibrillar Powder/ Liquid Solution	Organoleptic
Coliforms	< 100 cfu/g	USP Chapter <61>
Microbial Count	< 1000 cfu/g	USP Chapter <61>
Molds and Yeast	< 100 cfu/g	USP Chapter <61>
Heavy Metals	< 2.4 ppm	AOAC 993.14 Mod.
Collagen Purity	> 95% (**)	SDS-Page Electrophoresis
Collagen Identity	> 98% (^)	FTIR Spectrometry
Collagen Microstructure	Fibrillar	Optical Microscopy (40x)
Collagen Concentration	> 950 mg/g	Hydroxyproline Assay
Cytotoxicity	Non-Cytotoxic	ANSI/AAMI/ISO 10993-5:2009/(R)2014
Acute Systemic Toxicity	Non-Toxic	ANSI/AAMI/ISO 10993-11:2017(E)
Irritation and Skin Sensitization	Non-Irritant	ANSI/AAMI/ISO 10993-10:2010/(R)2014

(\*) Typical Characterization and Testing for Seallagen Type-I Collagen. For specification details for your collagen product and/or your lot number, please refer to the COA provided with your order.

(\*\*) >95% collagen contained with alpha ( $\alpha_1$ ,  $\alpha_2$ ), beta ( $\beta$ ) and gamma ( $\gamma$ ) bands for Seallagen Type-I Collagen

(^)>98% FTIR spectra matching the intensity bands corresponding type-I collagen (Riaz et al. 2018) for Seallagen Type-I Collagen

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**Storage/Stability:** The product is stored at 2-8°C and ships on frozen gel packs. Do not freeze. The expiration date is listed on the certificate of analysis for each specific lot. The expiration date is applicable when product is handled and stored as directed.

### **Precautions and Disclaimer**

The 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: Use these recommendations as guidelines to determine the optimal coating conditions for your culture systems.

1. Remove required quantity of collagen from the bottle and dispense into a dilution vessel.
2. Dilute Seallagen (or Seallagen-MCT) in water to ~50 to 100 ug/ml (~1:30). A 0.1 to 0.5 M HCl or HOAc solution may also be used.
3. Swirl contents gently until material is completely mixed.
4. Add appropriate amount of diluted Seallagen material to the culture surface ensuring the entire surface is coated.
5. Incubate at room temperature, covered, for 1-2 hours. Aspirate any remaining material. Alternatively, incubate at room temperature until surface is dry.
6. Rinse coated surfaces carefully with sterile medium or PBS, avoid scratching surfaces.
7. 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**

1. Slowly add 1 part of chilled 10X PBS or 10X culture media to 8 parts of chilled collagen solution with gently 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 2-8°C.
5. To form gel, warm to 37°C. Allow approximately 90 to 120 minutes for gel formation.