

# AC Marine Collagen PF Efficacy Data

**Code:** 20739  
**INCI Name:** Soluble Collagen  
**CAS #:** 9015-54-7  
**EINECS #:** 310-296-6

Type of Study	Results
<p><b>Hydration Analysis</b></p>	<p>The results indicate that <b>AC Marine Collagen</b> is capable of increasing stratum corneum moisturization values after 2 weeks. <b>AC Marine Collagen</b> may be added to formulations to effectively improve epidermal hydration.</p>
<p><b>Combability Assay</b></p>	<p>In comparison to the control shampoo, the shampoo that contained 5.0% AC Marine Collagen PF increased the sensorial perception of wet combing ease by 85.0%, whereas the control was rated as only improving combability by 25.0% for wet hair. When assessing dry hair combability, participants rated the control as increasing combability by 40.0% and the test material as increasing dry combability by 50.0%.</p>
<p><b>Moisturization Analysis</b></p>	<p>The results indicate that <b>AC Marine Collagen PBF</b> is capable of increasing stratum corneum moisturization values after 1 hour. <b>AC Marine Collagen PBF</b> may be added to anhydrous systems to effectively increase epidermal moisture.</p>
<p><b>Sirius Red Fast Green Assay</b></p>	<p>As shown in figure 1, <b>AC Marine Collagen PF</b> (code 20739) exhibited positive collagen synthesis activity. The increase in collagen production may lead to improvement in the dermal-epidermal junction integrity as well as an improved scaffolding matrix. For these reasons, we can assume <b>AC Marine Collagen PF</b> (code 20739) is suitable for cosmetic applications designed to boost collagen synthesis to aid in providing a younger and healthier complexion.</p>

**ORAC Assay**

**AC Marine Collagen** was designed to provide hydrating and conditioning properties. With the present study we can confirm that this unique ingredient is not only capable of providing functional benefits but it is also capable of providing potent antioxidant benefits when added to cosmetic applications.



# AC Marine Collagen *In-vivo* Hydration Analysis

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## Abstract:

Different mechanisms are frequently employed to improve epidermal hydration. A common method utilizes a combination of humectants and conditioners to hydrate and moisturize the skin; however marine proteins have been observed to have superior hydration properties. It is believed that **AC Marine Collagen** may effectively help hydrate the skin and plump the skin as they come in contact with water, which may be absorbed from the dermis to hydrate the stratum corneum. Measurements of epidermal impedance are typically used to determine changes in relative hydration levels.

## Materials and Methods:

10 (m/f) subjects between the ages of 22 and 64 were used to determine hydration improvements using an o/w emulsion that contained **AC Marine Collagen**. The DPM 9003 and XPRT Software purchased from NOVA Technology Corporation were used to obtain and analyze the initial stratum corneum hydration values on the volar forearm for each subject. The initial stratum corneum values measured prior to the application of the test product were used as a hydrolyzed collagen baseline to compare the values obtained following the application of the test product. Impedance measurements were taken 2 weeks after the test material was applied.

## Results:

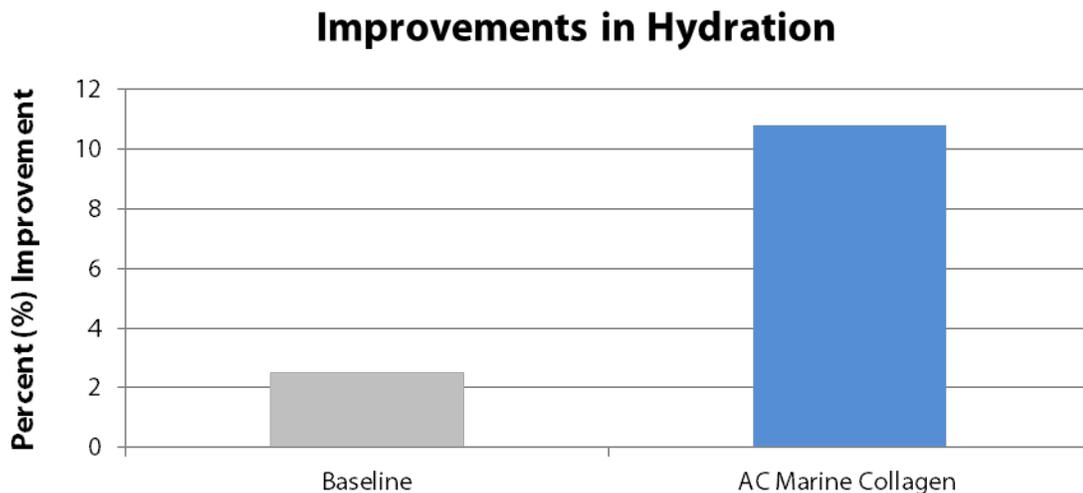


Figure 1: Percent improvement in hydration following application of **AC Marine Collagen**.

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# AC Marine Collagen *In-vivo* Hydration Analysis

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## Discussion:

The results indicate that **AC Marine Collagen** is capable of increasing stratum corneum moisturization values after 2 weeks. **AC Marine Collagen** may be added to formulations to effectively improve epidermal hydration.

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# Increase in Hair Combability AC Marine Collagen PF

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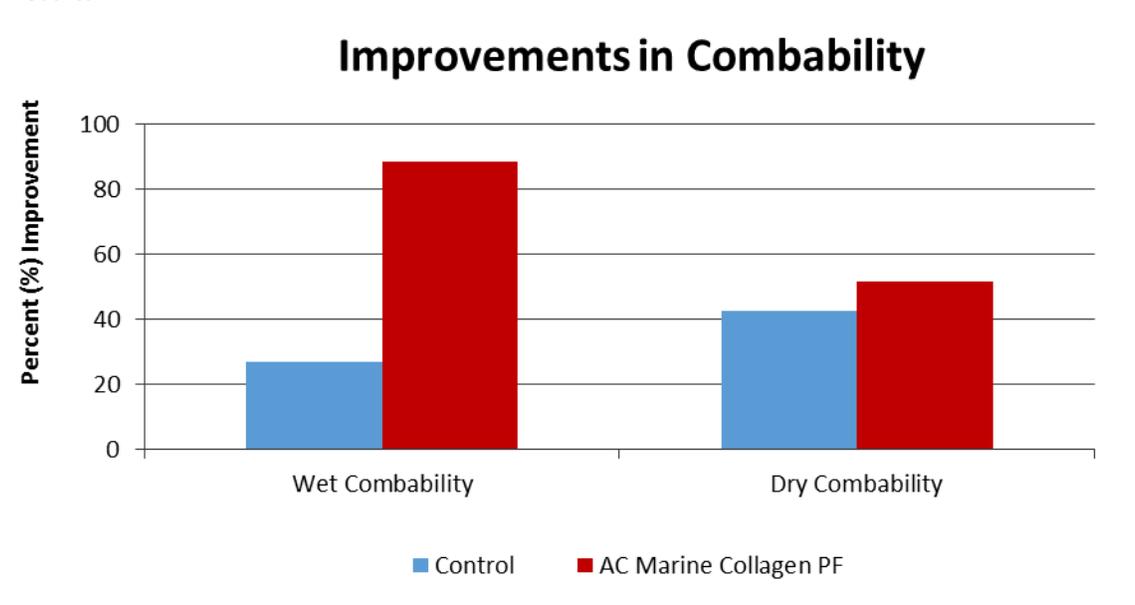
## Abstract:

AC Marine Collagen PF is intended to increase the manageability and combability of the hair. Hair is not alive; although the body naturally produces sebum to condition and protect hair it does not repair damage. The cuticle is the outermost layer of hair; it consists of protein chains that form a scale-like pattern along the entire hair shaft. The cuticle is easily damaged through regular hair maintenance regimens including, shampooing, drying, combing and styling. Hair is also damaged when exposed to heat, UV rays, dyes and permanent wave products. Damaged hair is often categorized as being dry, brittle, dull and unmanageable.

## Materials and Method:

A panel of 10 men and women between the ages of 18 and 36 was assembled. The subjects then had half of their hair shampooed with a control and the other half shampooed with a product containing 5.0% AC Marine Collagen PF. Subjects were asked to comb out their wet hair, rating each side on a scale of 1 – 10 for combability ease. One (1) being the most difficult and ten (10) being the easiest. Each subject’s hair was then dried with a blow dryer and they were again asked to rate the combability of each side of their head on a scale of 1 – 10.

## Results:



## Discussion:

In comparison to the control shampoo, the shampoo that contained 5.0% AC Marine Collagen PF increased the sensorial perception of wet combing ease by 85.0%, whereas the control was rated as only improving combability by 25.0% for wet hair. When assessing dry hair combability, participants rated the control as increasing combability by 40.0% and the test material as increasing dry combability by 50.0%.

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# AC Marine Collagen PBF In-Vivo Moisturization Analysis

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## Abstract:

Different mechanisms are frequently employed to improve epidermal moisturization. A fairly common method utilizes a combination of humectants and conditioners to hydrate and moisturize the skin; however marine proteins have been observed to have superior moisturizing properties. It is believed that **AC Marine Collagen PBF** may effectively help moisturize the skin and plump the skin as they come in contact with water, which may be absorbed from the dermis to hydrate the stratum corneum. Measurements of epidermal impedance are typically used to determine changes in relative hydration levels.

## Materials and Methods:

11 (m/f) subjects between the ages of 22 and 64 were used to determine moisturization improvements using an anhydrous skin care product that contained **AC Marine Collagen PBF**. The DPM 9003 and XPRT Software purchased from NOVA Technology Corporation were used to obtain and analyze the initial stratum corneum hydration values on the volar forearm for each subject. The initial stratum corneum values measured prior to the application of the test product were used as a hydrolyzed collagen baseline to compare the values obtained following the application of the test product. Impedance measurements were taken 1 hour after the test material was applied. The formula used to calculate the percent increase in moisture is:

## Results:

### Increase in Moisturization using AC Marine Collagen PBF

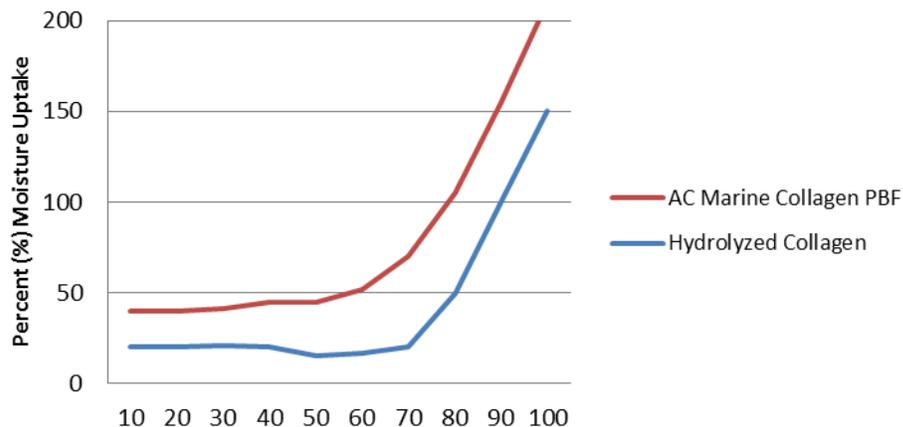


Figure 2: Percent increase in moisturization following application of **AC Marine Collagen PBF**.

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# AC Marine Collagen PBF *In-Vivo* Moisturization Analysis

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## Discussion:

The results indicate that **AC Marine Collagen PBF** is capable of increasing stratum corneum moisturization values after 1 hour. **AC Marine Collagen PBF** may be added to anhydrous systems to effectively increase epidermal moisture.

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# Sirius Red/Fast Green Collagen Analysis

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**Tradename:** AC Marine Collagen PF

**Code:** 20739

**Lot #:** 36265

**CAS #:** 9015-54-7

**Test Request Form #:** 947

**Sponsor:** Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092

**Study Director:** Erica Segura

**Principle Investigator:** Meghan Darley

**Test Performed:**

Sirius Red/Fast Green Collagen Assay

## Introduction

Collagen is the main protein of connective tissues, such as skin, bone, tendon and ligament, and the most abundant protein in mammals. Collagen accounts for nearly 25% to 35% of the total human protein content. Collagen is a long, fibrous protein that forms bundles called fibers giving structure and support to cells and tissues. Collagen has great tensile strength and is responsible for skin's elasticity and, therefore, its degradation leads to wrinkles that accompany aging.

Sirius Red/Fast Green Collagen Assay was conducted to assess the changes in collagen synthesis by **AC Marine Collagen PF** treated *in vitro* cultured human dermal fibroblasts.

## Assay Principle

Sirius Red is a unique dye that binds specifically to the helical structure of types I through V collagen, while Fast Green binds to non-collagenous proteins. These two dyes work in conjunction to provide a semi-quantitative method of determining amounts of collagen and non-collagenous proteins in a sample. After staining a sample the dyes are easily extracted and have optical density (OD) absorptions at 540nm (Sirius Red) and 605nm (Fast Green). Protein concentrations are calculated through equations with OD values.

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## Sirius Red/Fast Green Collagen Analysis

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### Materials

- |                                  |  |
|----------------------------------|--|
| <b>A. Kit:</b>                   | Sirius Red/Fast Green Collagen Kit (Chondrex; 9046)  |
| <b>B. Incubation Conditions:</b> | 37°C at 5% CO <sub>2</sub> and 95% Relative Humidity (RH)  |
| <b>C. Equipment:</b>             | Forma Humidified Incubator, ESCO Biosafety Laminar Flow Hood, Synergy HT Microplate Reader; Pipettes           |
| <b>D. Cell Line:</b>             | Normal Human Dermal Fibroblasts (Invitrogen; C-004-5C)   |
| <b>E. Media/Buffers:</b>         | Complete and Serum-Free Dulbecco's Modified Eagle Medium (DMEM); Phosphate Buffered Saline (PBS)               |
| <b>F. Culture Plate:</b>         | Falcon Flat Bottom 24-Well Tissue Culture Treated Plates   |
| <b>G. Reagents:</b>              | Ascorbic Acid-2-Glucose(AA2G) (100µM); Insulin Growth Factor-1 (IGF-1) (50ng/mL); Glacial Acetic Acid, Ethanol |
| <b>H. Other:</b>                 | Sterile Disposable Pipette Tips; Wash Bottles  |

### Methods

Human dermal fibroblasts were seeded into 24-well tissue culture plates and allowed to grow to confluency in complete DMEM. 1%, 0.1%, and 0.01% concentrations of **AC Marine Collagen PF** were added to the serum-free DMEM and incubated with fibroblasts for 24 hours. AA2G and IGF-1 were used as positive controls.

Media was removed from wells containing adherent fibroblasts and the cells were washed with PBS. 500µl of a cooled 95% ethanol/5% glacial acetic acid solution was added to the wells and incubated for 10 minutes at room temperature. 200µL of the Sirius Red/Fast Green dye solution was added to the fixed cell layer and incubated at room temperature for 30 minutes. The dye solution was removed from the cell layer and washed with water. 1mL of extraction solution was added for color extraction. The optical density was read at 540nm and 605nm on the Synergy HT Microplate Reader.

The protein concentrations of **AC Marine Collagen PF** treated-fibroblasts were determined by calculations based on the optical density measurements and expressed in µg.

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## Results

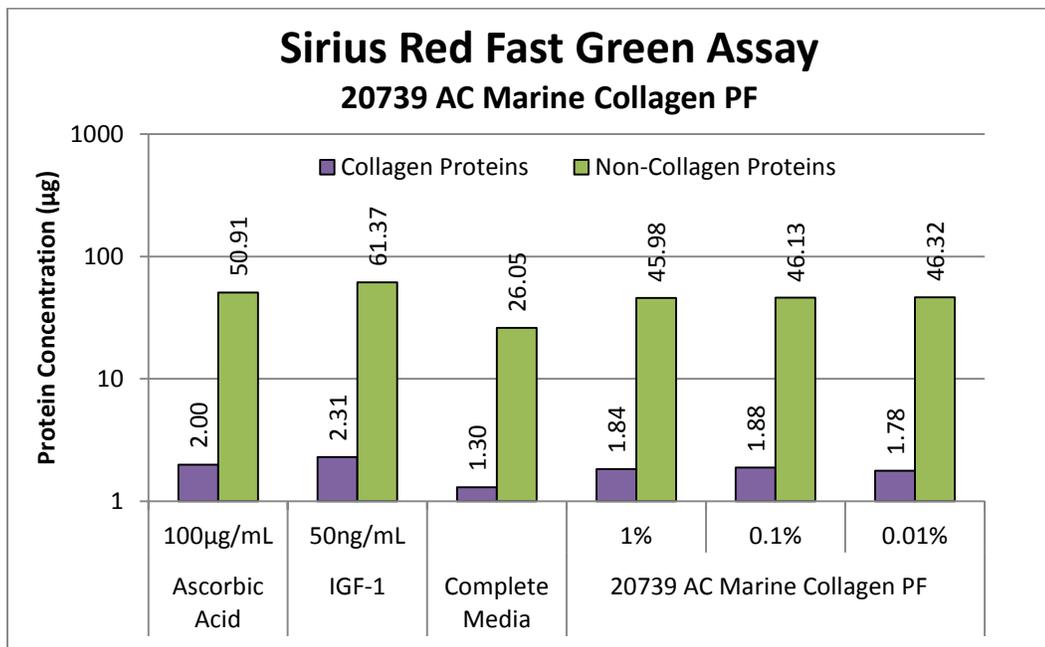
The data obtained from this study met criteria for a valid assay and the positive and negative controls performed as anticipated.

**AC Marine Collagen PF** elicited positive effects on collagen synthesis.

Collagen concentration is calculated by the following formula:

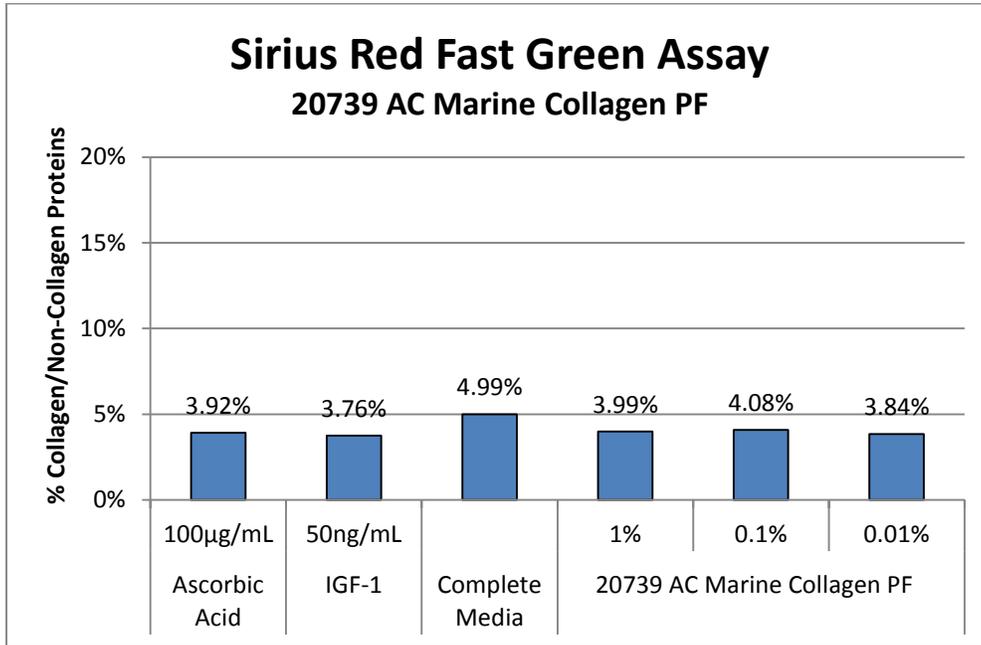
$$\text{Collagen } (\mu\text{g}) = \frac{[OD\ 540 - (OD\ 605 \times 0.291)]}{0.0378}$$

$$\text{Non Collagen Protein } (\mu\text{g}) = \frac{OD\ 605}{0.00204}$$



**Figure 1:** Collagen and non-collagen protein concentrations

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**Figure 2:** Percent collagen compared to non-collagen proteins

## Discussion

As shown in figure 1, **AC Marine Collagen PF** (code 20739) exhibited positive collagen synthesis activity. The increase in collagen production may lead to improvement in the dermal-epidermal junction integrity as well as an improved scaffolding matrix. For these reasons, we can assume **AC Marine Collagen PF** (code 20739) is suitable for cosmetic applications designed to boost collagen synthesis to aid in providing a younger and healthier complexion.

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# Oxygen Radical Absorbance Capacity (ORAC) Assay

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**Tradename:** AC Marine Collagen

**Code:** 20598

**CAS #:** 9015-54-7

**Test Request Form #:** 294

**Lot #:** 27906

**Sponsor:** Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092

**Study Director:** Erica Segura

**Principle Investigator:** Meghan Darley

**Test Performed:**

Oxygen Radical Absorbance Capacity (ORAC)

## Introduction

Reactive oxygen species (ROS) are generated by normal cellular processes, environmental stresses, and UV irradiation. ROS are detrimental to cellular structures and functional molecules (i.e DNA, proteins, lipids) as they act as strong oxidizing agents or free radicals. The oxygen radical absorbance capacity (ORAC) assay is a standard method used to assess antioxidant capacity of physiological fluids, foods, beverages, and natural products. The assay quantitatively measures a sample's ability to quench free radicals that have the potential to react with and damage cellular components.

Oxygen Radical Absorbance Capacity (ORAC) assay was conducted to assess the antioxidant capacity of **AC Marine Collagen**.

## Assay Principle

This assay is based upon the effect of peroxy radicals generated from the thermal decomposition of 2, 2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) on the signal intensity from the fluorescent probe, fluorescein, in the presence of an oxygen radical absorbing substance. The degree of change is indicative of the amount of radical damage and the presence of antioxidants results in an inhibition in the free radical damage to the fluorescein. The antioxidant protection of the sample can be calculated by comparing it to a set of known standards. Trolox®, a water soluble vitamin E analog, with known antioxidant capabilities is used in this ORAC assay as the standard for measuring the antioxidant capacity of unknown substances. ORAC values, expressed in  $\mu\text{M}$  of Trolox® equivalents (TE), are calculated using the area under the curves (AUC) of the test product, Trolox®, and the control materials. Trolox equivalency is used as the benchmark for antioxidant capacity of mixtures since it is difficult to measure individual components.

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# Oxygen Radical Absorbance Capacity (ORAC) Assay

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## Materials

- A. Equipment:** Synergy H1 Microplate reader (BioTek Instruments, Winooski, VT); Gen5 software (BioTek Instruments, Winooski, VT); Pipettes
- B. Buffers:** 75mM Potassium Phosphate (pH 7.4); Deionized H<sub>2</sub>O
- C. Reagents:** 2,2'-Azobis(2-methylpropionamide) dihydrochloride (AAPH) (153mM); 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox®); Fluorescein Sodium Salt (4nM)
- D. Preparation:** Pre-heat (37°C) Synergy H1 Microplate reader; Prepare Trolox® standards, sample dilutions, fluorescein solution, and AAPH.
- E. Microtitre Plates:** Corning 96 Well Black Side/Clear Bottom Microplates

## Methods

Solutions of **AC Marine Collagen** and Trolox® (positive control) were prepared in 75mM potassium phosphate buffer. Materials were prepared at three different concentrations/dilutions. Trolox® was used as a reference for antioxidant capacity and prepared at a concentrations ranging from 12.5µM to 200µM in 75mM potassium phosphate buffer.

For the ORAC assay, 25µL of test material and Trolox® were combined with 150µL of fluorescein in 75mM potassium phosphate buffer and incubated in the Synergy HT Microplate reader at 37°C for 30 minutes. At the end of the incubation period, 25µL of AAPH were pipetted into each well. Fluorescent measurements were then taken every 2 minutes for approximately 2 hours at an excitation wavelength of 485nm and an emissions wavelength of 520nm.

The AUC and Net AUC values of the standards and samples were determined using Gen5 2.0 Data Reduction Software using the below equations:

$$AUC = 0.5 + \frac{R2}{R1} + \frac{R3}{R1} + \frac{R4}{R1} + \dots + \frac{Rn}{R1} \rightarrow \text{Where } R \text{ is fluorescence reading}$$

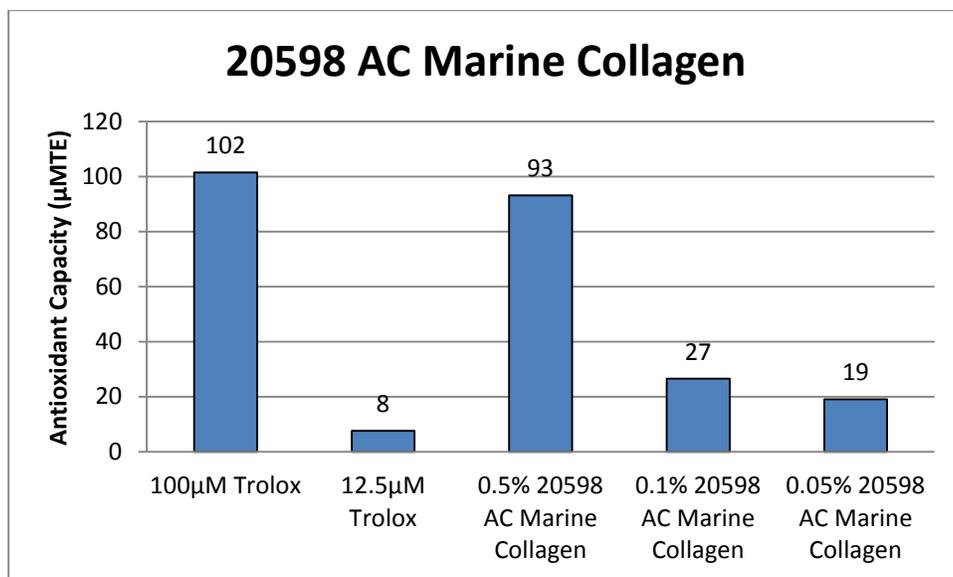
$$\text{Net AUC} = AUC_{\text{sample}} - AUC_{\text{blank}}$$

The standard curve was obtained by plotting the Net AUC of different Trolox® concentrations against their concentration. ORAC values of samples were then calculated automatically using the Gen5 software to interpolate the sample's Net AUC values against the Trolox® standard curve. ORAC measurements for the test material were expressed in micro moles Trolox® equivalents (µMTE), where 1 ORAC unit is equal to 1 µMTE.

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## Results

**AC Marine Collagen** began exhibiting antioxidant activity at a 0.05% concentration.



**Figure 1:** Antioxidant capacities

## Discussion

As shown in figure 1, **AC Marine Collagen** exhibited antioxidant activity comparable to 100µM Trolox®. The antioxidant capacity of **AC Marine Collagen** increased as the concentration increased, as a result we can assure that its ability to minimize oxidative stress is dose dependent.

**AC Marine Collagen** was designed to provide hydrating and conditioning properties. With the present study we can confirm that this unique ingredient is not only capable of providing functional benefits but it is also capable of providing potent antioxidant benefits when added to cosmetic applications.