

**Tradename:** AC Soluble Elastin PF

**Code:** 20595PF

**CAS #:** 91080-18-1

**Test Request Form #:** 4795

**Lot #:** NC181203-I

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

**Study Director:** *Maureen Drumwright*

**Principle Investigator:** *Jennifer Goodman*

**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. Specifically, it 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.

Accordingly, a Sirius Red/Fast Green Collagen Assay was conducted to assess the *in vitro* effect of **AC Soluble Elastin PF** to trigger collagen synthesis in 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 samples, the dyes are easily extracted and have optical density (OD) absorptions at 540 nm (Sirius Red) and 605 nm (Fast Green). Collagen concentrations are calculated through equations with OD values.

## Materials

- A. Kit:** Sirius Red/Fast Green Collagen Kit (Chondrex; 9046)\*
- B. Incubation Conditions:** 37°C, 5% CO<sub>2</sub>, and 95% relative humidity (RH)
- C. Equipment:** Forma humidified incubator; ESCO biosafety laminar flow hood; Synergy HT Microplate reader; Pipettes; Light microscope
- D. Cell Line:** Normal Human Dermal Fibroblasts (ATCC; PCS-201-012)\*
- E. Media/Buffers:** Fibroblast Basal Medium (PCS-201-030)\*; Fibroblast Growth Kit (PCS-201-041)\*; Phosphate Buffered Saline (PBS)
- F. Reagents:** Sirius Red/Fast Green dye solution (Chondrex; 9046)\*; Extraction solution (Chondrex; 9046)\*; Ascorbic Acid-2-Glucose (AA2G) (100 µM); Insulin Growth Factor-1 (IGF-1) (50 ng/mL); Glacial Acetic Acid; Ethanol
- G. Culture Plate:** Flat Bottom 24-Well Tissue Culture Treated Plates
- H. Other:** Sterile disposable pipette tips

\*Or suitable alternatives, subject to change without notice based off vendor availability

## Methods

Human dermal fibroblasts were seeded into 24-well tissue culture plates and allowed to grow to confluency in complete media (CM). 0.01%, 0.1%, and 1.0% concentrations of **AC Soluble Elastin PF** were diluted in serum-free CM and incubated with fibroblasts for 24 hours. AA2G (100 µM) and IGF-1 (50 ng/mL) were diluted in serum-free CM and utilized as positive controls.

Media was removed from wells containing adherent fibroblasts and the cells were washed with PBS. 500 µL of a cooled 95% ethanol/5% glacial acetic acid solution was added to the wells and incubated for 10 minutes at room temperature. 200 µL 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 PBS. 1 mL of extraction solution was added for color extraction and optical density (OD) was read at 540 nm and 605 nm on a Synergy HT Microplate Reader.

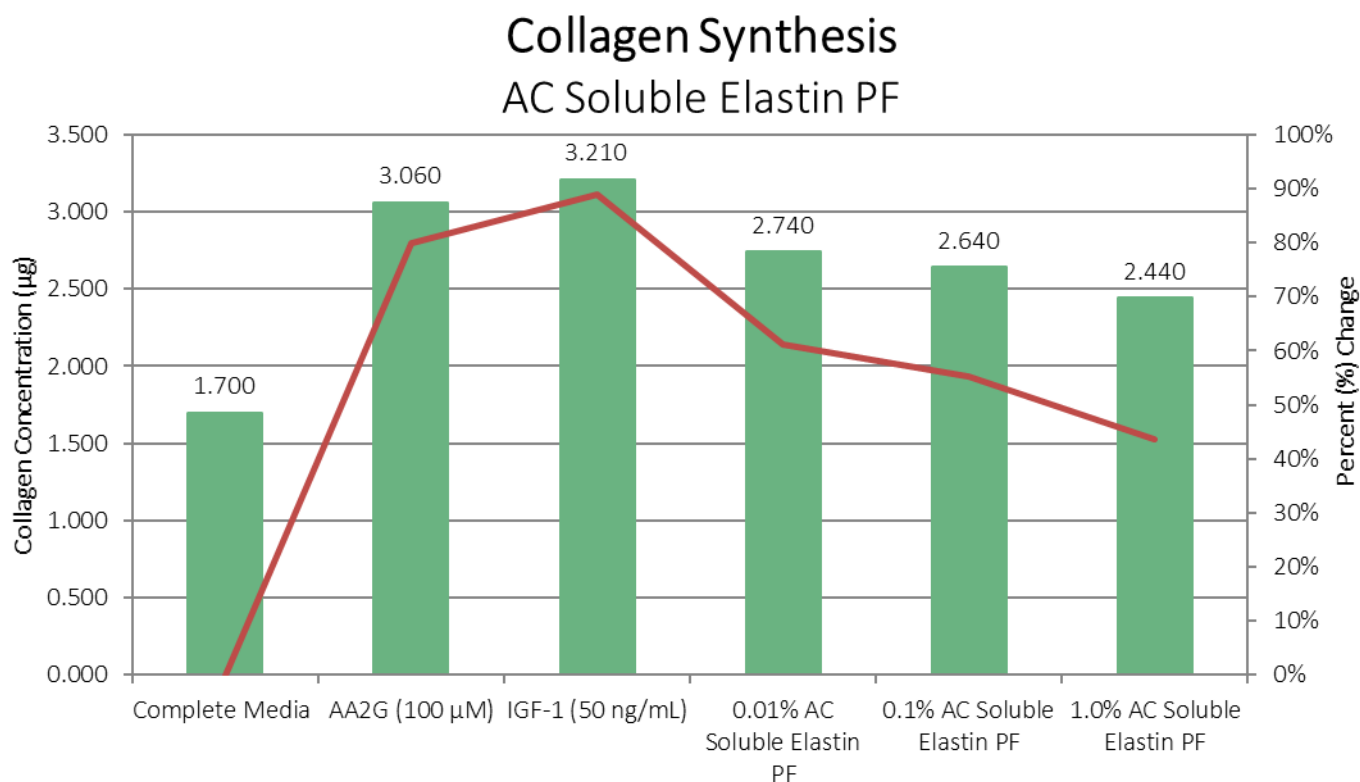
The collagen and non-collagen protein concentrations of fibroblasts were determined by the following calculations and expressed in µg:

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

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

## Results

The data obtained from this study met criteria for a valid assay and the positive controls performed as anticipated. Compared to untreated fibroblasts, AA2G (100 µM) and IGF-1 (50 ng/mL) increased collagen concentrations. Fibroblasts treated with **AC Soluble Elastin PF** at 0.01%, 0.1%, and 1.0% demonstrated increased collagen synthesis compared to untreated fibroblasts.



**Figure 1.** The effect of **AC Soluble Elastin PF** on collagen concentrations in dermal fibroblasts.

## Discussion

As shown in Figure 1, fibroblasts incubated with Ascorbic Acid-2-Glucose (AA2G) and Insulin Growth Factor-1 (IGF-1), both known to stimulate collagen synthesis, exhibited increases in collagen synthesis of 80% and 89% compared to untreated fibroblasts, respectively. These data demonstrate collagen in fibroblasts is dynamic and can be manipulated with exogenous compounds.

Similarly, fibroblasts treated with **AC Soluble Elastin PF** at 0.01%, 0.1%, and 1.0% demonstrated potent increases in collagen synthesis of 61%, 55% and 44%, respectively, compared to untreated fibroblasts (\*Please note that when interpreting *in vitro* studies, a 1.0% concentration is comparable to a 100% dose in application. This high dosage can account for slightly decreased viability and efficacy *in vitro* and is included for comparison purposes.). Overall, these data demonstrate **AC Soluble Elastin PF** activates collagen synthesis in dermal fibroblasts.

Collectively, increases in collagen production may lead to improvements in dermal-epidermal junction integrity as well as an improved scaffolding matrix. These data indicate **AC Soluble Elastin PF** stimulates collagen synthesis which may assist cosmetic applications designed to enhance collagen deposition and provide a younger and healthier dermal complexion.