Pro-Collagen I α 1 ELISA

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Tradename: AC Collagen Prepeptide PF

Code: 20452PF

CAS #: 92113-31-0

Test Request Form #: 9852

Lot #: 8961000

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Drumwright*
Principal Investigator: *Daniel Shill*

Test Performed:

Pro-Collagen I α 1 Enzyme-Linked Immunosorbent Assay (ELISA)

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. Therefore, its degradation leads to wrinkles that accompany aging. Accordingly, a Pro-Collagen I α 1 ELISA was conducted to assess the *in vitro* effect of **AC Collagen Prepeptide PF** on the extracellular release of Pro-Collagen I α 1 from human dermal fibroblasts.

Assay Principle

The Pro-Collagen I α 1 ELISA Kit operates by mixing an affinity tag labeled capture antibody with a reporter conjugated detector antibody that binds to Pro-Collagen I α 1. After Pro-Collagen I α 1 is labeled, an immobilized complex is formed upon binding to anti-tag antibodies coating the wells. Unbound materials are removed during washing steps, and adding 3,3',5,5'-tetramethylbenzidine (TMB) Development Solution generates a blue color that is catalyzed by horseradish peroxidase (HRP). Adding Stop Solution to samples finalizes the color change from blue to yellow and absorbance is measured. The signal generated is proportional to the amount of bound Pro-Collagen I α 1 and concentrations are calculated. Cell culture supernatants (media) from all conditions are collected to determine secreted levels of Pro-Collagen I α 1.

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Materials

A. Kit:	Human Pro-Collagen I α 1 ELISA Kit (ab210966)*
B. Incubation Conditions:	37°C, 5% CO_2, 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 Neo-Natal Dermal Fibroblasts (ATCC; PCS-201-010)*
E. Media/Buffers:	Fibroblast Basal Medium (PCS-201-030)*; Fibroblast Growth Kit (PCS-201-041)*; Antibody Diluent CPI (ab210966)*; 10X Wash Buffer PT (ab210966)*; Sample Diluent NS (ab210966)*
F. Reagents:	Insulin Growth Factor-1 (IGF-1) (ab210966)*; Pro-Collagen I α 1 Recombinant Protein (ab210966)*; 10X Pro-Collagen I α 1 Capture Antibody (ab210966)*; 10X Pro-Collagen I α 1 Detector Antibody (ab210966)*; TMB Development Solution (ab210966)*; Stop Solution (ab210966)*; Washer Buffer PT (ab210966)*
G. Culture Plate:	Flat Bottom 12-Well Tissue Culture Treated Plates; Pre-Coated 96 Well Microplate (12 x 8 well strips) (ab210966)*
H. Software:	Excel Analysis ToolPak (Microsoft)
I. Other:	Sterile Disposable Pipette Tips; Wash Bottles; 15 mL Conical Tubes, 1.5 mL Microcentrifuge Tubes

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

Methods

Human dermal neo-natal fibroblasts were seeded into a 12-well tissue culture plate and grown to 80%-90% confluency in complete media (CM). 0.01%, 0.1% and 1.0% concentrations of **AC Collagen Prepeptide PF** in CM were added to cells and placed at 37°C. IGF-1 (50 ng/mL) was added to CM as a positive control. After 48 hours, media and fibroblast lysates were collected according to the manufacturer's instructions and utilized in the Human Pro-Collagen I α 1 ELISA Kit (ab210966). All conditions were measured in duplicate.

Standards were prepared for the media samples, ranging in concentrations from 0 pg/mL to 2,000 pg/mL. After adding 50 µL of standards and samples to the appropriate wells, 50 µL of the Antibody Cocktail was added to all wells. Following a one-hour incubation at room temperature, all wells were washed three times with 350 µL of 1x Wash Buffer PT and 100 µL of TMB Development Solution was added to each well. After a 10-minute incubation in the dark, 100 µL of Stop Solution was added to each well. The optical density was read at 450 nm on a Synergy HT Microplate Reader.

Assays were repeated three separate times with each sample run in duplicate. Duplicates for each replicate were averaged, and the average of all three experiments is displayed. Data was analyzed using a one-way ANOVA with statistical significance accepted at $p \leq 0.05$. A standard curve was created by reducing the data and generating a linear curve fit. The secreted Pro-Collagen I α 1 concentrations of **AC Collagen Prepeptide PF**-treated fibroblasts were determined by extrapolation from the standard curve and expressed in pg/mL. Percent changes in Pro-Collagen I α 1 are relative to CM and were calculated with the following formula:

$$Percent\ Change = \frac{Pro\text{-}Collagen\ I\ \alpha\ 1\ Concentration_{Sample} - Pro\text{-}Collagen\ I\ \alpha\ 1\ Concentration_{CM}}{Pro\text{-}Collagen\ I\ \alpha\ 1\ Concentration_{CM}} \times 100$$

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Results

The data obtained from this study met criteria for a valid assay and the positive control performed as anticipated. Compared to untreated fibroblasts, IGF-1 increased secreted Pro-Collagen I α 1 concentrations. Fibroblasts treated with **AC Collagen Prepeptide PF** at 0.01%, 0.1%, and 1.0% enhanced the levels of secreted Pro-Collagen I α 1 above the values of CM alone.

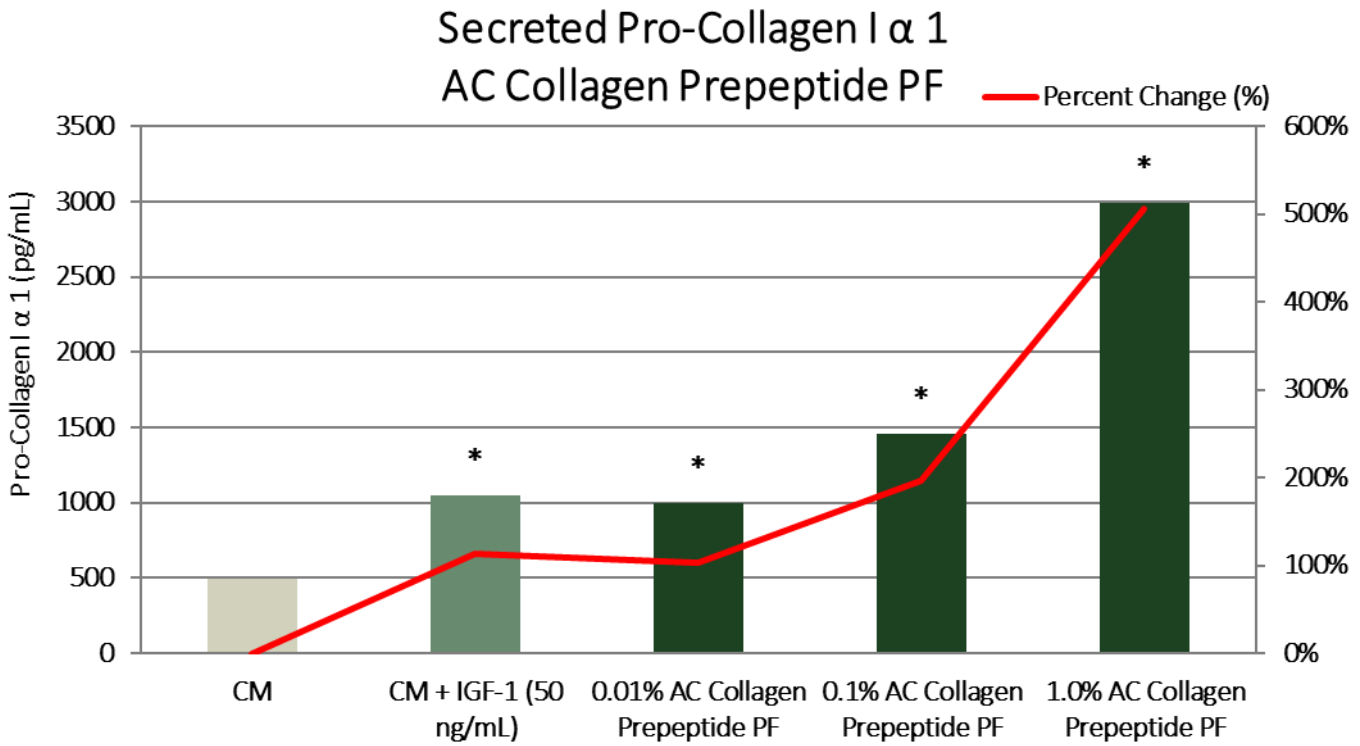

Figure 1. Pro-Collagen I α 1 secreted by fibroblasts. * indicates significance ($p \leq 0.05$) compared to untreated fibroblasts.

Table 1. Results from one-way ANOVA Statistical Analysis of Pro-Collagen I α 1 Secreted Compared to Untreated Fibroblasts. * indicates significance ($p \leq 0.05$) compared to untreated fibroblasts.

	IGF-1	0.01% **AC Collagen Prepeptide PF**	0.1% **AC Collagen Prepeptide PF**	1.0% **AC Collagen Prepeptide PF**
P-value	0.001*	0.002*	< 0.001*	< 0.001*

Discussion

As shown in Figure 1, fibroblasts exposed to IGF-1 demonstrated a 113% increase in Pro-Collagen I α 1 secretion compared to untreated fibroblasts (Table 1). This data demonstrates the levels of Pro-Collagen I α 1 released from fibroblasts can be augmented with compounds known to increase collagen synthesis. Similarly, treatment with **AC Collagen Prepeptide PF** at 0.01%, 0.1%, and 1.0% demonstrated 103%, 196%, and 506% increases in the levels of Pro-Collagen I α 1 released from fibroblasts, respectively, compared to untreated fibroblasts. These data demonstrate **AC Collagen Prepeptide PF** has the ability to augment fibroblast Pro-Collagen I α 1 secretion.

Increases in Pro-Collagen I α 1 secretion enhance collagen deposition leading to improved scaffolding matrix and dermal-epidermal junction integrity. Accordingly, **AC Collagen Prepeptide PF** appears to be suitable for cosmetic applications designed to aid in providing a younger and healthier complexion by augmenting collagen synthesis.