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AC Collagen Prepeptide PF Efficacy Data

Code: 20452PF
INCI Name: Tripeptide-29
CAS #: 92113-31-0
EINECS #: 295-635-

Name of Study	Type of Study	Results
Cellular Viability Assay	*in-vitro*	AC Collagen Prepeptide was tested to evaluate its effects on the viability of normal human dermal fibroblasts (NDHF). At a concentration of 0.01% AC Collagen Prepeptide PF increases cellular viability by 11.04%. It can therefore be concluded that at normal use concentrations AC Collagen Prepeptide PF enhances cellular viability.
Increase in Collagen Synthesis	*in-vitro*	The results indicate that after a period of 48 hours, 3% AC Collagen Prepetide PF is capable of increasing type I collagen synthesis by 400%. AC Collagen Prepeptide PF is an ideal ingredient to use in cosmetic applications were potent anti-wrinkle and anti-aging benefits are desired.

info@activeconceptsllc.com • +1 (704)-276-7100 • Fax: +1 (704)-276-7101

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info@activeconceptsllc.com • +1 (704)-276-7100 • Fax: +1 (704)-276-71

Cellular Viability Assay Analysis

Tradename: AC Collagen Prepepitde PF

Code: 20452PF

Lot #: 34715

CAS #: 92113-31-0

Test Request Form #: 966

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
Cellular Viability Assay

Introduction

The cellular viability assay is useful for quantitatively measuring cell-mediated cytotoxicity, cell proliferation and mitochondrial metabolic activity. Increased metabolism in a cell indicates ample cellular respiration and adenosine triphosphate (ATP) production. ATP is the molecular energy of cells and is required in basic cell function and signal transduction. A decrease is ATP levels indicates cytotoxicity and decreased cell function while an increase in ATP levels indicates healthy cells.

The cellular viability assay was conducted to assess the ability of **AC Collagen Prepeptide PF** to increase cellular metabolic activity in cultured dermal fibroblasts.

Assay Principle

The assay utilizes a nonfluorescent dye, resazurin, which is converted to a fluorescent dye, resorufin, in response to chemical reduction of growth medium from cell growth and by respiring mitochondria. Healthy cells that are in a proliferative state will be able to easily convert resazurin into resorufin without harming the cells. This method is a more sensitive assay than other commonly used mitochondrial reductase dyes such as MTT. An increase in the signal generated by resazurin-conversion is indicative of a proliferative cellular state.

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Materials

A.	**Kit:**	PrestoBlue™ Cell Viability Reagent (Invitrogen, A13261)
B.	**Incubation Conditions:**	37°C at 5% CO_2 and 95% relative humidity (RH)
C.	**Equipment:**	Forma humidified incubator; ESCO biosafety laminar flow hood; Light microscope; Pipettes
D.	**Cell Line:**	Normal Human Dermal Fibroblasts (NHDF) (Lonza; CC-2511)
E.	**Media/Buffers:**	Dulbecco's Modified Eagle Medium (DMEM); Penicillin-Streptomycin (50U-50mg/mL); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS)
F.	**Culture Plate:**	Falcon flat bottom 96-well tissue culture treated plates
G.	**Reagents:**	PrestoBlue™ reagent (10X)
H.	**Other:**	Sterile disposable pipette tips

Methods

Human dermal fibroblasts were seeded into 96-well tissue culture plates and allowed to grow to confluency in complete DMEM. A 10-fold serial dilution was performed resulting in **AC Collagen Prepeptide PF** concentrations on 1%, 0.1%, and 0.01% in complete DMEM and incubated with fibroblasts for 24 hours.

Ten microliters of viability reagent was added to 90µL of cell culture media in culture wells and a fluorometric measurement was taken at 560nm for excitation and 590nm for emission.

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Results

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

AC Collagen Prepeptide PF exhibited positive effects on cell metabolism.

Cellular metabolism results are shown as mean fluorescence units (MFU) and expressed as percentage change, calculated by the below equation:

$$Percent\ (\%)Change = \frac{MFU_{Control} - MFU_{Sample}}{MFU_{Control}} \times 100$$

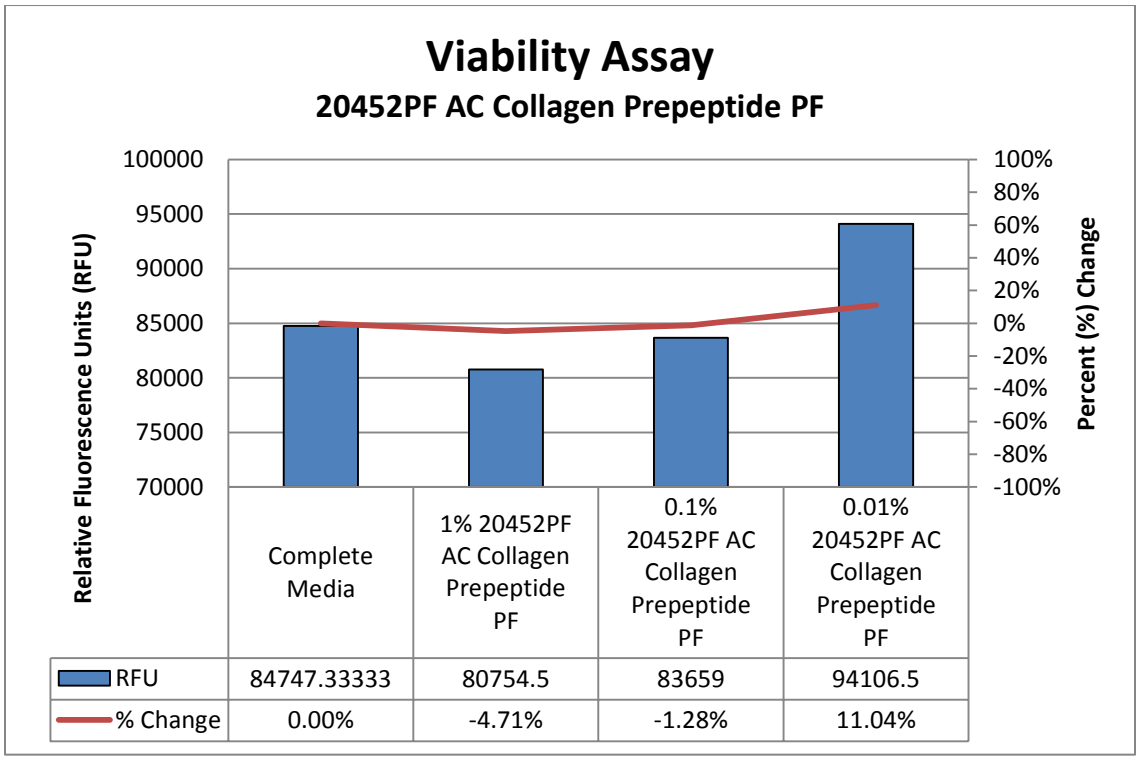

Figure 1: Cellular Metabolism of **AC Collagen Prepeptide PF**-treated fibroblasts expressed in terms of percent of control.

Discussion

In this study, **AC Collagen Prepeptide PF** (code 20452PF) was tested to evaluate its effects on the viability of normal human dermal fibroblasts (NDHF). At a concentration of 0.01% **AC Collagen Prepeptide PF** (code 20452PF) increases cellular viability by 11.04%. It can therefore be concluded that at normal use concentrations **AC Collagen Prepeptide PF** (code 20452PF) enhances cellular viability.

Increase in Collagen I Synthesis
AC Collagen Prepeptide PF

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Tradename: AC Collagen Prepeptide PF

Code: 20452PF

CAS #: 92113-31-0

Test Request Form #: 946

Lot #: 32112P

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
Increase in Collagen I Synthesis

Abstract

The conserved amino acid sequence of Glycine-Proline-Hydroxyproline is believed to increase *in-vivo* fibroblast activity for collagen synthesis. AC Collagen Prepeptide PF is a pure (>99.7%) synthetic polypeptide consisting of the Glycine-Proline-Hydroxyproline conserved sequence. Quantitative analysis is intended to demonstrate AC Collagen Prepeptide PF's efficacy to stimulate collagen type I production.

Materials and Methods

Adult human dermal fibroblasts were obtained from Cell Applications, Inc. The fibroblasts were cultured in 100-mm diameter petri dishes using Eagle's MEM supplemented with 9% FCS, ascorbic acid, nonessential amino acids, amphotericin B (1µg/ml), streptomycin (100µg/ml), penicillin (100U/ml) and Earle's salts, which were obtained from Gibco Laboratories. The cells were grown to confluence. The fibroblasts were then treated with 3% (30µg/ml) of AC Collagen Prepeptide PF. To determine the effect of AC Collagen Prepeptide PF on collagen production, cells were plated at $1x10^5$ per well and cultured for 48 hours. Media from the wells was then collected to measure collagen I using a Capture ELISA kit (MDBiosciences). This kit was used according to the manufacturer's instructions.

Increase in Collagen I Synthesis
AC Collagen Prepeptide PF

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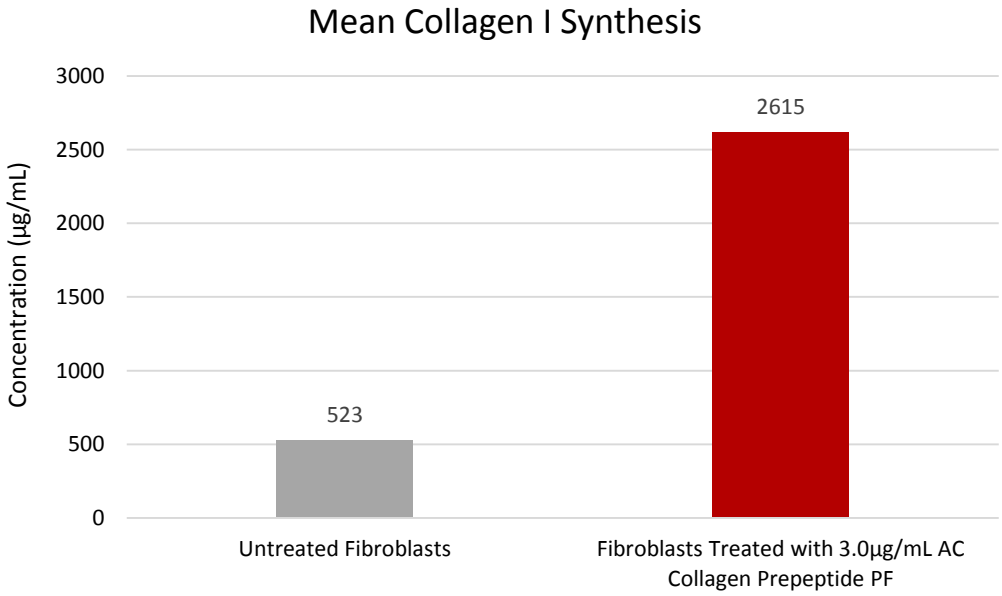
Results

	Mean Collagen Concentration (µg/ml)
Untreated Fibroblasts	523
Fibroblasts treated with 3% AC Collagen Prepeptide PF	2615
Percent increase when using 3% AC Collagen Prepeptide PF	400%



Discussion

The results indicate that after a period of 48 hours, **3% AC Collagen Prepetide PF** is capable of increasing type I collagen synthesis by 400%.

Given that collagen is the primary building block that provides structural support to the skin, improving its concentration can help minimize the appearance of fine lines and wrinkles. This will achieve a smoother and younger looking complexion.

For this reason **AC Collagen Prepeptide PF** is an ideal ingredient to use in cosmetic applications were potent anti-wrinkle and anti-aging benefits are desired.

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