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Tomorrow's Vision... *Today!*®

AC Sebum Control Enzyme PF Efficacy Data

Code: 20395PF
INCI Name: Butylene Glycol & Water & Spiraea Ulmaria Extract
CAS #: 107-88-0 & 7732-18-5 & 84775-57-5
EINECS #: 203-529-7 & 231-791-2 & 283-866-3

Name of Study	Results
Antimicrobial Activity Assay	**AC Sebum Control Enzyme PF** is able to sufficiently inhibit the growth of both *Staphylococcus epidermidis* and *Propionibacterium acnes*. **AC Sebum Control Enzyme PF** is also able to reduce the accumulation of sebum within the pilo-sebaceous channels that assists in the proliferation of skin bacteria and ultimately leads to irritation and acne.
Inhibition of 5-α Reductase Assay	**AC Sebum Control Enzyme PF** inhibits 5α-reductase at all tested levels and is dose-dependent, as the highest dose level is most effective. 0.075% **AC Sebum Control Enzyme PF** decreased 5α-reductase mRNA by 18%; 0.10% **AC Sebum Control Enzyme PF** decreased 5α-reductase mRNA by 52%; and 0.15% **AC Sebum Control Enzyme PF** decreased 5α-reductase mRNA by 69%.
Astringent Properties Assay	**AC Sebum Control Enzyme PF** decreased the lipid index in 100% of our volunteers and it lowered 73% of volunteers down to normal skin lipid levels. Therefore, **AC Sebum Control Enzyme PF** has adequate astringent properties and sufficiently lowers sebum secretion down to normal skin levels.
Visual Astringency Assay	According to the results, **AC Sebum Control Enzyme PF** may dramatically reduce the number of visual sebum spots, thus improving the overall appearance and health of the skin.

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Cellular Viability Assay	**AC Sebum Control Enzyme PF** was tested to evaluate its effects on the viability of normal human dermal fibroblasts (NDHF). At a concentration of 1% **AC Sebum Control Enzyme PF** increases cellular viability by almost 19%. It can therefore be concluded that at normal use concentrations **AC Sebum Control Enzyme PF** enhances cellular viability.

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AC Sebum Control Enzyme PF

Code: 20395PF

Abstract

The purpose of this study was to determine the effects of **AC Sebum Control Enzyme PF** *in-vitro* on antimicrobial activity. Two skin bacteria that are responsible for acne were tested: *Staphylococcus epidermidis* and *Propionibacterium acnes*.

Materials and Methods

1 ml of each bacterial solution (*Staphylococcus epidermidis* and *Propionibacterium acnes*) containing ~10^4 germs/ml was placed on a Petri dish and covered with agar solution. Wells were punched in the agar and filled with 1%, 2%, 3%, and 4% **AC Sebum Control Enzyme PF**, and results were compared to a control (50/50 gallic/ellagic acid). The Petri dishes were incubated at 30°C for 48 hours for Staphylococcus and 37°C for 72 hours for Propionibacterium. The diameter of the plaques is proportional to the levels of antimicrobial activity.

Results

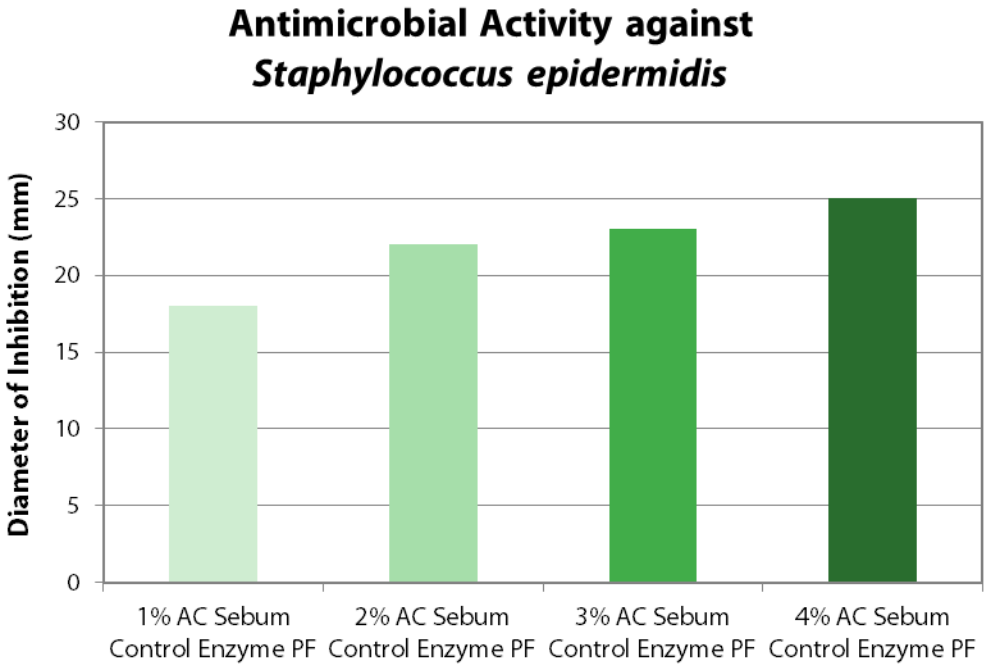

Figure 1. The antimicrobial activity of **AC Sebum Control Enzyme PF** on *Staphylococcus epidermidis*.

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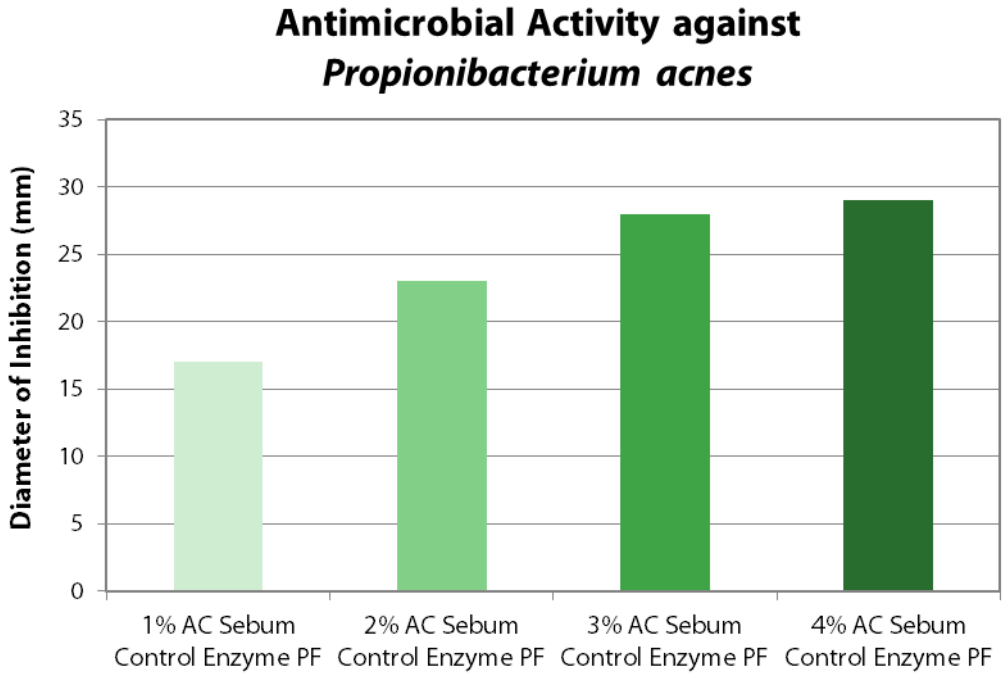

Figure 2. The antimicrobial activity of **AC Sebum Control Enzyme PF** on *Propionibacterium acnes*.

Discussion

AC Sebum Control Enzyme PF is able to sufficiently inhibit the growth of both microbials, *Staphylococcus epidermidis* and *Propionibacterium acnes*. **AC Sebum Control Enzyme PF** is able to reduce the accumulation of sebum within the pilo-sebaceous channels that assists in the proliferation of skin bacteria and ultimately leads to irritation and acne. Thus by inhibiting the growth of these skin bacteria, **AC Sebum Control Enzyme PF** should act as a deterrent against problem skin.

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AC Sebum Control Enzyme PF

Code: 20395PF

Abstract

The purpose of this study was to determine the effects of **AC Sebum Control Enzyme PF** on the inhibition of 5α-reductase in human keratinocytes. The enzyme 5α-reductase is responsible for the production of the androgen hormone dihydrotestosterone, which stimulates the sebaceous glands to produce sebum. To determine the levels of 5α-reductase, we measured mRNA levels by Reverse Transcription Polymerase Chain Reaction (RT-PCR) and running an agarose gel. According to the results, we found that the application of **AC Sebum Control Enzyme PF** inhibits 5α-reductase production, and thus controls the production of sebum within the sebaceous glands.

Materials and Methods

Active Concepts' technical staff incubated human keratinocytes for 72 hours at 35°C in an atmosphere of 5% CO_2 in a medium containing 0.075% **AC Sebum Control Enzyme PF**, 0.10% **AC Sebum Control Enzyme PF**, and 0.15% **AC Sebum Control Enzyme PF**, and these were compared to a control. After incubation, total RNA was extracted from each sample and subjected to RT-PCR, using complementary nucleotides to obtain the mRNA. mRNA was run on an agarose gel, and quantities were determined according to band intensity versus the mRNA of β-actin, an internal standard. The intensity of the bands was determined according to a computer software program, and the results were expressed as a ratio in relation to the internal standard.

Results

Results were determined according to the following formula:

$$\% \text{ Decrease in mRNA} = \frac{(S/I)}{(C/I)} \times 100$$

where the parameters are as follows:

S = AC Sebum Control Enzyme PF band intensity
I = Internal Standard (β-actin) band intensity
C = Control band intensity

Inhibition of 5α-reductase

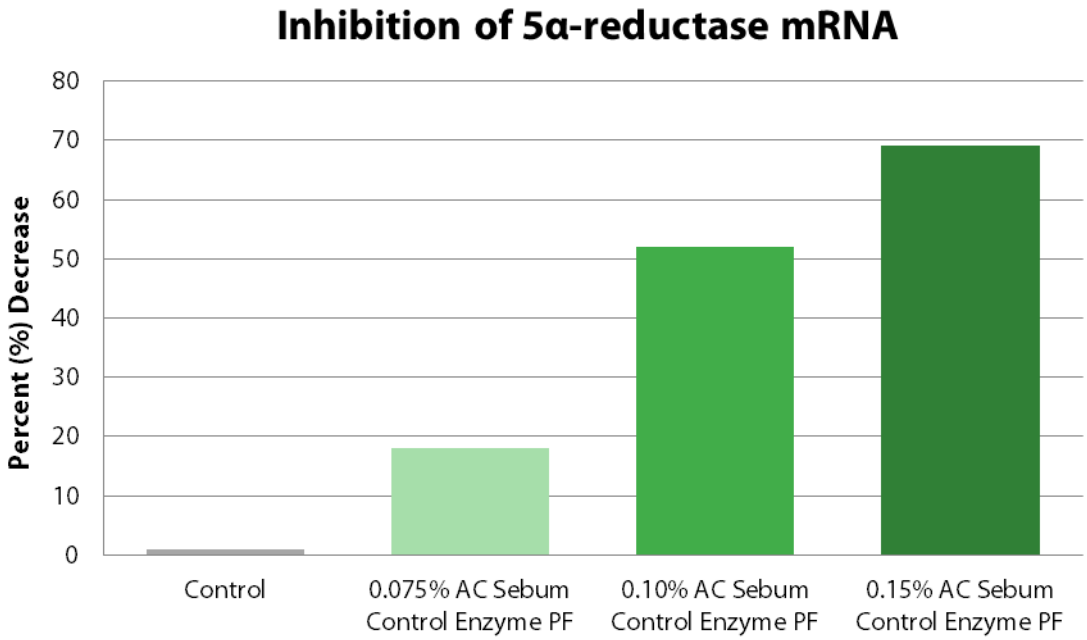

Figure 1. The effects of **AC Sebum Control Enzyme PF** on the inhibition of 5α-reductase mRNA in human keratinocytes.

Discussion

mRNA expression of 5α-reductase correlates to the activity of dihydrotestosterone, and so plays a role in the regulation and production of sebum. According to the results, **AC Sebum Control Enzyme PF** inhibits 5α-reductase at all tested levels and is obviously dose-dependent, as the highest dose level is most effective. 0.075% **AC Sebum Control Enzyme PF** decreased 5α-reductase mRNA by 18%; 0.10% **AC Sebum Control Enzyme PF** decreased 5α-reductase mRNA by 52%; and 0.15% **AC Sebum Control Enzyme PF** decreased 5α-reductase mRNA by 69%. Therefore because 5α-reductase regulates the production of sebum in the sebaceous glands, by inhibiting 5α-reductase **AC Sebum Control Enzyme PF** should also inhibit the production of sebum that would eventually be secreted onto the stratum corneum.

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AC Sebum Control Enzyme PF

Code: 20395PF

Abstract

The purpose of this study was to determine the *in vivo* astringent effect of 5% **AC Sebum Control Enzyme PF** versus a placebo. Photometric measurement was used to determine the lipid index (the refractory index that correlates with the concentration of lipids on the surface of the skin). Measurements were taken before use and then again after 30 days of twice daily application. According to the results, **AC Sebum Control Enzyme PF** may reduce the amount of sebum secretion on the stratum corneum.

Materials and Methods

15 volunteers with oily skin between the ages of 25 and 46 years old took part in this study. Symmetrical skin areas on the forehead of each volunteer were determined and photometric measurement by a Sebumeter® was used to establish the lipid index of each individual. A placebo was applied twice daily in one area, and 5% **AC Sebum Control Enzyme PF** was applied on the other identical area. Measurements were taken before use (at D0) and then again after 30 days (at D30) of twice daily application.

Results

The results were determined at D0 and D30 and the lipid index for each individual volunteer is as follows:

		Placebo			5% AC Sebum Control Enzyme PF	
Volunteer	D0	D30	D0-D30	D0	D30	D0-D30
1	244	226	18	244	180	64
2	302	273	29	302	173	129
3	219	224	-5	219	124	95
4	248	203	45	248	156	92
5	215	176	39	215	197	18
6	311	284	27	311	201	110
7	242	189	53	242	188	54
8	199	203	-4	199	102	97
9	192	149	43	192	126	66
10	228	193	35	228	193	35
11	236	164	72	236	164	72
12	205	179	26	205	179	26
13	294	195	99	294	195	99
14	238	185	53	238	132	106
15	251	204	47	251	118	133
Mean	**242**	**203**	**38**	**242**	**162**	**80**

Table 1. The change in lipid index due to the astringent properties of **AC Sebum Control Enzyme PF**.

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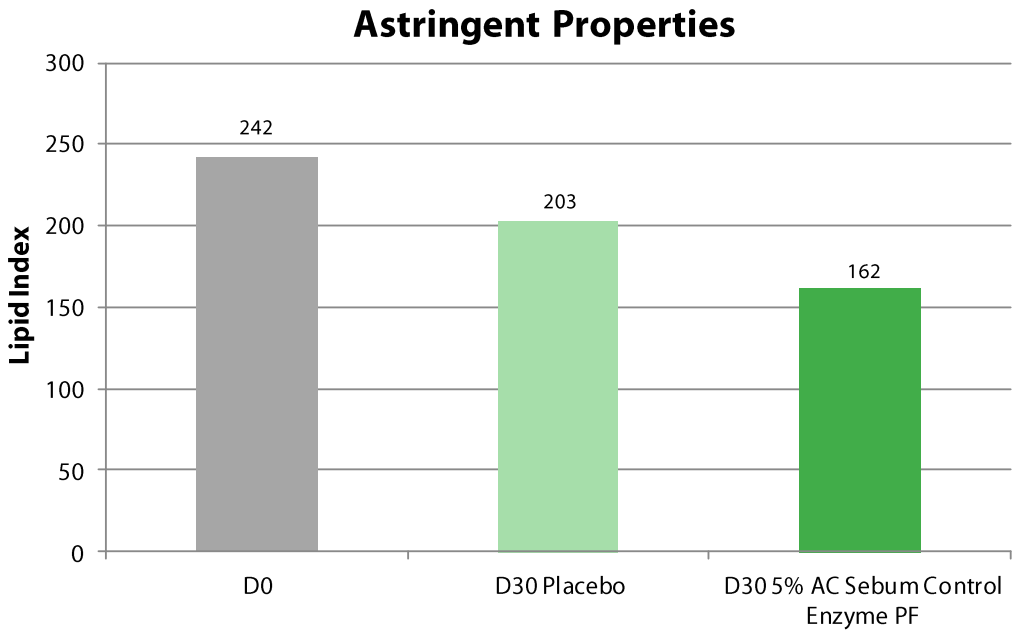

Figure 1. The decrease in sebum secretion due to the astringent properties of **AC Sebum Control Enzyme PF**.

Discussion

The lipid index indicates that a measurement above 190 depicts oily skin; thus 100% of our volunteers were considered to have oily skin at D0. According to the results, **AC Sebum Control Enzyme** decreased the lipid index in 100% of our volunteers and it lowered 73% of volunteers down to normal skin lipid levels. Therefore **AC Sebum Control Enzyme** may have adequate astringent properties and sufficiently lower sebum secretion down to normal skin levels.

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AC Sebum Control Enzyme PF

Code: 20395PF

Abstract

The purpose of this study was to determine the *in-vivo* visual astringency of 5% **AC Sebum Control Enzyme PF**. Sebutape® was used to determine the sebum on the surface of the skin. After cleaning the skin and removing all topical oils with alcohol, the Sebutape® was applied for 20 minutes and then analyzed with a digital image analyzer to determine the number and intensity of sebum spots. According to the results, **AC Sebum Control Enzyme PF** may reduce the amount of sebum secretion on the stratum corneum.

Materials and Methods

5 volunteers between the ages of 22 and 36 years old took part in this study. The face of each volunteer was cleaned with alcohol, and Sebutape® was applied to the forehead for 20 minutes at D0. (Sebutape® is composed of hydrophobic and hydrophilic polymers that have the capacity to absorb lipids.) **AC Sebum Control Enzyme PF** was applied twice daily for 30 days, and then on D30 the face of each volunteer was cleaned with alcohol, and Sebutape® was applied to the forehead of each volunteer for 20 minutes. The surface area and intensity of sebum spots on the tape represents the quantity of lipids present.

Results

The results were determined at D0 and D30 and the number of sebum spots for each individual volunteer is as follows:

Volunteer	**D0**	**D30**	**D0-D30**
1	103	24	79
2	88	41	47
3	95	62	33
4	78	30	48
5	91	29	62
Mean	**91**	**37**	**54**

Table 1. The change in the number of sebum spots due to the astringency of **AC Sebum Control Enzyme PF**.

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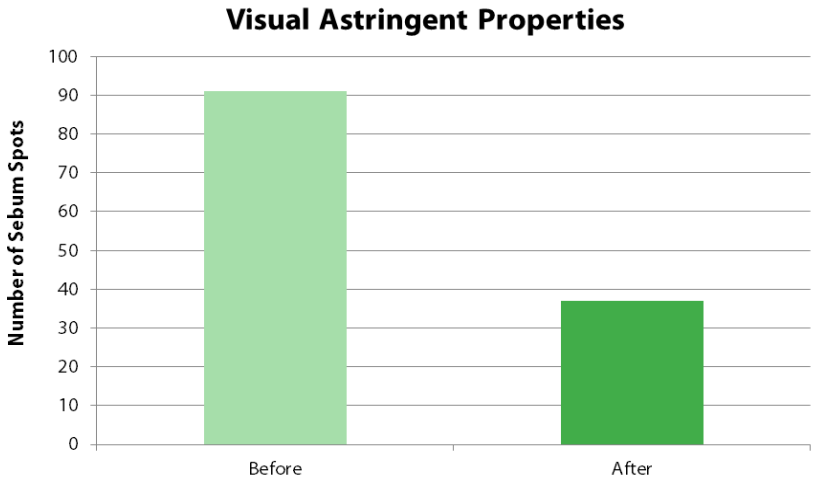

Figure 1. The decrease in the number of sebum spots due to the astringency of **AC Sebum Control Enzyme PF**.

Discussion

The number of sebum spots on the Sebutape® directly shows the visual change in astringency on the surface of the skin due to the application of **AC Sebum Control Enzyme PF**. According to the results, **AC Sebum Control Enzyme PF** may dramatically reduce the number of visual sebum spots, thus improving the overall appearance and health of the skin.

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Cellular Viability Assay Analysis

Tradename: AC Sebum Control Enzyme PF

Code: 20395PF

CAS #: 107-88-0 & 7732-18-5 & 84775-57-5

Test Request Form #: 941

Lot #: NC140123-A

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 Sebum Control Enzyme 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 Sebum Control Enzyme 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 Sebum Control Enzyme PF had 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:

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

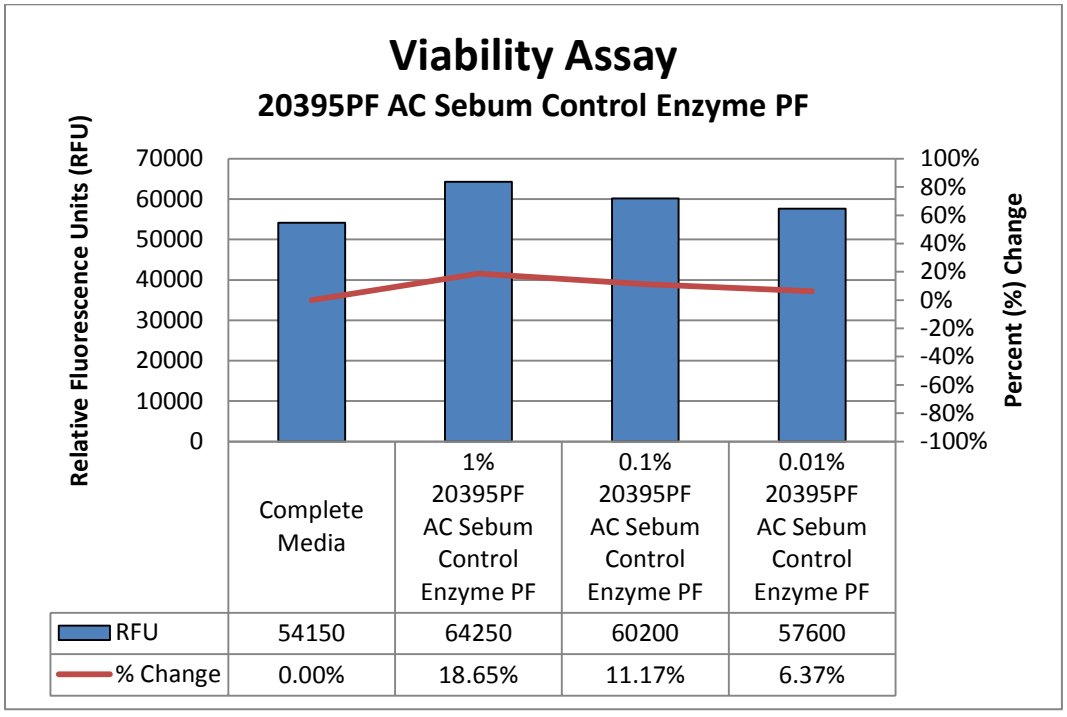

Figure 1: Cellular Metabolism of **AC Sebum Control Enzyme PF**-treated fibroblasts expressed in terms of percent of control.

Discussion

In this study, **AC Sebum Control Enzyme PF** (code 20395PF) was tested to evaluate its effects on the viability of normal human dermal fibroblasts (NDHF). At a concentration of 1% **AC Sebum Control Enzyme PF** (code 20395PF) increases cellular viability by almost 19%. It can therefore be concluded that at normal use concentrations **AC Sebum Control Enzyme PF** (code 20395PF) enhances cellular viability.