ACB Modified Pomegranate Enzyme Efficacy Data

Code: 20440
INCI Name: Lactobacillus/Punica Granatum Fruit Ferment Filtrate
CAS #: 84961-57-9
EINECS #: 284-646-0

Name of Study	Results
Cellular Renewal Assay	**ACB Modified Pomegranate Enzyme PF** is capable of increasing cellular renewal when compared to the untreated control.
Cellular Viability Assay	**ACB Modified Pomegranate Enzyme PF** was tested to evaluate its effects on the viability of normal human dermal fibroblasts (NDHF). At a concentration of 1% **ACB Modified Pomegranate Enzyme PF** increases cellular viability by 5.45% It can therefore be concluded that at normal use concentrations **ACB Modified Pomegranate Enzyme PF** enhances cellular viability.
Protein Stability Assay	After six months of stability testing, the overall protein content of **ACB Modified Pomegranate Enzyme PF** remains consistent. This indicates that this product is stable. Slight variation of results is due to the degree of error associated with this particular methodology.

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Cellular Renewal Assay

Tradename: ACB Modified Pomegranate Enzyme PF

Code: 20440PF

CAS #: 84961-57-9

Test Request Form #: 3421

Lot #: 52257P

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Test Performed:
Dansyl Chloride

Abstract

ACB Modified Pomegranate Enzyme PF was evaluated for its ability to accelerate cell renewal by means of a traditional Dansyl Chloride protocol.

Materials and Methods

A 5% Dansyl Chloride was prepared by dispersing Dansyl Chloride 95% (Sigma) in petrolatum. Approximately 0.2 g of the ointment was applied to three 2cm x 2cm locations on the volar forearm of 12 (M/F) subjects between the ages of 20 and 45. The material was allowed to remain in place for 24 hours at which time any excess ointment was removed.

Two products were tested, with the remaining untreated site serving as the biological control. The products were applied in a randomized fashion. Approximately 50 μl of product was applied to the appropriate test site once per day. The sites were then examined daily under ultraviolet light (SL-3660 Long Wave Ultra Violet, Black Light Eastern Corp., Westbury, Long Island, NY) for fluorescence. The test was continued until no fluorescence was detectable at any site. The values listed reflect the average time for each product.

Results

Material	Concentration	Days	% Cell Renewal
Glycolic Acid	4%	16	28.6
Untreated Control	N/A	23	N/A
ACB Modified Pomegranate Enzyme PF	5%	17	26

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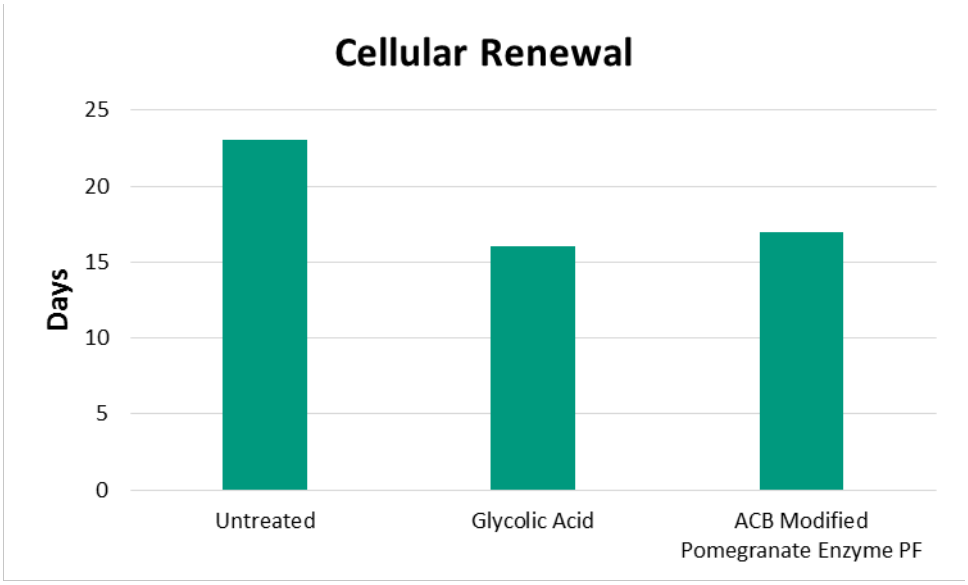

Discussion

The results indicate that **ACB Modified Pomegranate Enzyme PF** is capable of increasing cellular renewal by 26% when compared to the untreated biological control.

Cellular Viability Assay Analysis

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Tradename: ACB Modified Pomegranate Enzyme PF

Code: 20440PF

CAS #: 84961-57-9

Test Request Form #: 944

Lot #: 33755P

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

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 **ACB Modified Pomegranate 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 **ACB Modified Pomegranate 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.

ACB Modified Pomegranate Enzyme 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:

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

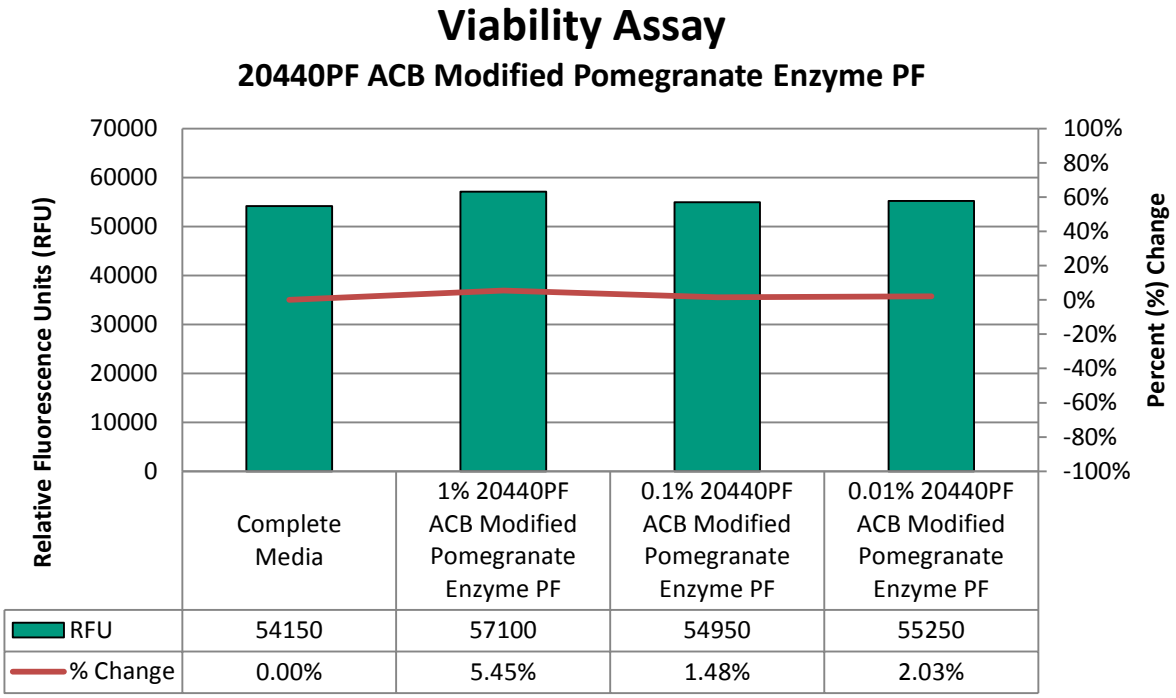

Figure 1. Cellular Metabolism of **ACB Modified Pomegranate Enzyme PF**-treated fibroblasts expressed in terms of percent of control.

Discussion

In this study, **ACB Modified Pomegranate Enzyme PF** was tested to evaluate its effects on the viability of normal human dermal fibroblasts (NDHF). At a concentration of 1.0% **ACB Modified Pomegranate Enzyme PF** increases cellular viability by 5.45% It can therefore be concluded that at normal use concentrations **ACB Modified Pomegranate Enzyme PF** enhances cellular viability.

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Protein Stability Assay

Tradename: ACB Modified Pomegranate Enzyme PF

Code: 20440PF

CAS #: 84961-57-9

Test Request Form #: 565

Lot #: 28182P

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

Test Performed:
Bradford Protein Assay to Determine Stability of Proteins

Introduction

The Bradford protein assay is a widely used spectroscopic analytical procedure used to measure the amount of protein in a solution.

The purpose of this study is to determine the initial protein content of **ACB Modified Pomegranate Enzyme PF**, then retest at set intervals to analyze the protein stability of the product.

Materials

1. Equipment
    a. UV/VIS Spectrophotometer
    b. Disposable Cuvettes
2. Reagents
    a. Bradford Reagent:
        i. Dissolve 100 mg Coomassie Brilliant Blue G-250 in 50 mL of 95% ethanol. Add 100 mL of 85% (w/v) phosphoric acid. Dilute to 1 L with DI water. When the dye is completely dissolved, filter through Whatman #1 filter paper. Store in a refrigerator.
    b. 0.15N NaCl Solution
    c. 0.5 mg/mL BSA (Bovine Serum Albumin) Solution

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Methods

1. Turn on spectrophotometer and run blank with DI water.
2. Into four separate, labeled test tubes, aliquot 5, 10, 15, and 20 µL of BSA solution. Add 0.15N NaCl to each until the volume of each tube is 100 µL.
3. Place the negative control solution (100 µL 0.15N NaCl) into a separate test tube.
4. To each of the 5 tubes, add 1 mL Bradford Reagent and vortex. Let stand at 20-25^{o}C for approximately 2 minutes.
5. Measure absorbance of each solution at 595 nm.
6. Generated a standard curve by plotting Absorbance versus protein concentration.
7. Repeat steps 1-6 using the test solution in place of the BSA solution.
8. Plot absorbance and use the standard curve to determine protein content.

Results

ACB Modified Pomegranate Enzyme PF

Initial Protein Content:	0.52 g/L
1 week:	0.49 g/L
2 weeks:	0.51 g/L
3 weeks:	0.54 g/L
4 weeks:	0.47 g/L
2 months:	0.48 g/L
3 months:	0.46 g/L
4 months:	0.50 g/L
5 months:	0.48 g/L
6 months:	0.49 g/L

Discussion

After six months of stability testing, the overall protein content of **ACB Modified Pomegranate Enzyme PF** remains consistent. This indicates that this product is stable. Slight variation of results is due to the degree of error associated with this particular methodology.