Active Concepts

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# Cellular Viability Assay Analysis

**Tradename:** ACB Modified Pomegranate Enzyme PF

**Code:** 20440PF

**CAS #:** 84961-57-9 & 1686112-10-6 (or) 84775-94-0 (or) 9015-54-7

**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 50mg/mL); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (50U- (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:

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

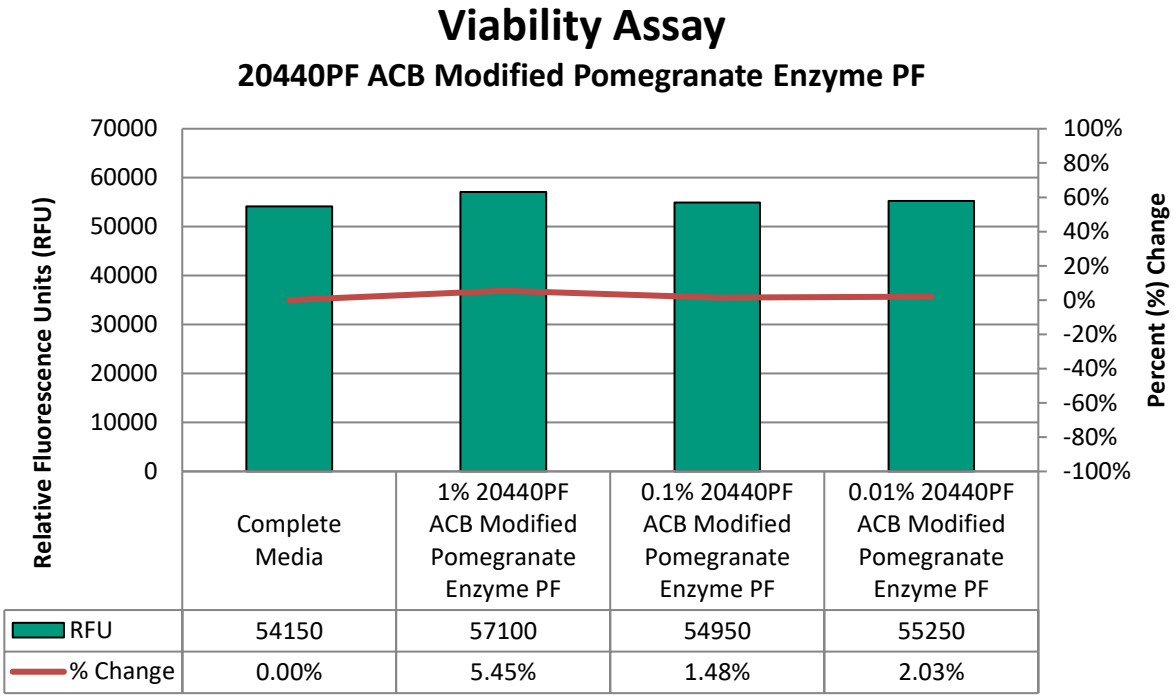


**Figure 1.** Cellular Metabolism of **ACB Modified Pomegranate Enzyme PF**-treated fibroblasts expressed in terms of percent of control.

## 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.