Cellular Viability Assay Analysis

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Tradename: AC ExoTone

Code: 60194

CAS #: 7732-18-5 & 85251-63-4 & 123465-35-0 (or) 8002-43-5

Test Request Form #: 10247

Lot #: N230421D

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Drumwright*
Principle Investigator: *Hannah Duckett*

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 in 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 ExoTone** 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, 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 Dermal Fibroblasts (NHDF) (Lonza; CC-2511)*
E. Media/Buffers:	Basal Medium (Fibrolife; LM-0001)*, 500 µg/mL Human Serum Albumins (Fibrolife; LS-1001)*, 0.6 µM Linoleic Acid (Fibrolife; LS-1001)*, 0.6 µg/mL (Fibrolife; LS-1001)*, 5.0 ng/mL Fibroblast Growth Factor (Fibrolife; LS-1002)*, 5 mg/mL Epidermal Growth Factor (Fibrolife; LS-1003)*, 30 pg/mL Transforming Growth Factor β-1 (Fibrolife; LS-2003)*, 7.5 mM L-Glutamine (Fibrolife; LS-1006)*, 1.0 µg/mL Hydrocortisone Hemisuccinate (Fibrolife; LS-1007)*, 50 µg/mL Ascorbic Acid (Fibrolife; LS-1005)*, 5.0 µg/mL Insulin (Fibrolife; LS-1004)*
F. Tissue Culture Plates:	Falcon flat bottom 96-well tissue culture treated plates
F. Reagents:	PrestoBlue™ reagent (10X)
G. Other:	Sterile disposable pipette tips

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

Methods

Human dermal fibroblasts were seeded into 96-well tissue culture plates and allowed to grow to confluency in complete serum-free media. A 10-fold serial dilution was performed resulting in **AC ExoTone** concentrations of 0.01% 0.1% and 1.0% in complete serum-free media and incubated with fibroblasts for 24 hours.

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

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

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

Results

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

AC ExoTone did not exhibit negative effects on cell metabolism.

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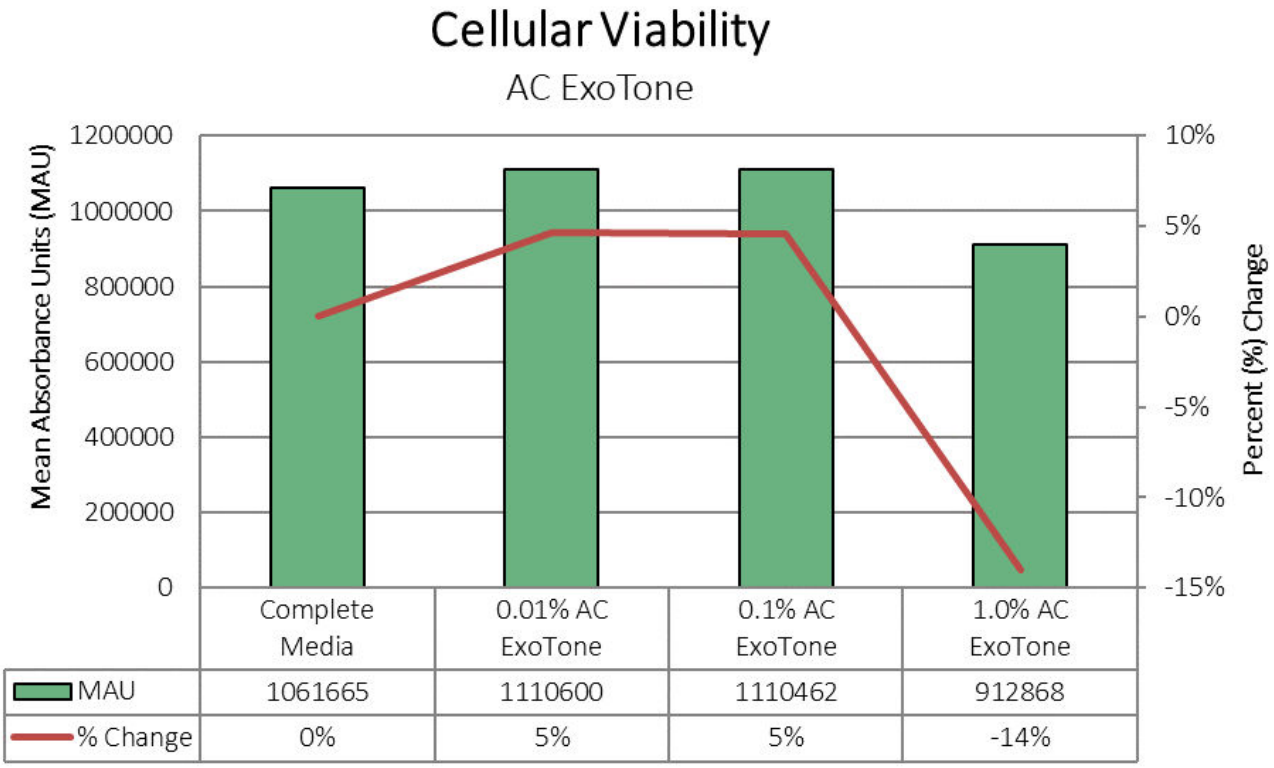

Figure 1. Cellular Metabolism of **AC ExoTone**-treated fibroblasts.

Discussion

In this study, **AC ExoTone (60194)** was tested to evaluate its effects on the viability of normal human dermal fibroblasts (NDHF). At concentrations of 0.01% and 0.1%, and 1.0%, **AC ExoTone**, nor the preservatives contained therein exhibited any inhibition of cell viability.

Please note that when interpreting *in vitro* studies, a 1.0% concentration is comparable to a 100% dose in application. This high dosage can account for slightly decreased viability and efficacy *in vitro* and is included for comparison purposes.

It can therefore be concluded that at normal use concentrations **AC ExoTone** is not cytotoxic.