Cellular Viability Assay Analysis

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Tradename: AC ExoEternol

Code: 60200

CAS #: 7732-18-5 & 68333-16-4 (or) 92128-79-5 & 90082-61-4 (or) 68132-21-8 & 123465-35-0 (or) 8002-43-5 & 68333-16-4 (or) 1686112-36-6 (or) 9015-54-7

Test Request Form #: 13036

Lot #: N250701O

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Daniel Shill*
Principal Investigator: *Hannah Stade*

Test Performed:
Cellular Viability Assay

Introduction

Cosmetic ingredients interact directly with dermal cells to provide a multitude of skin benefits. Assessing the impact of topical cosmetics on cellular homeostasis is an important screening step to avoid disrupting natural skin balance and normal skin barrier function. The cellular viability assay is useful for quantitatively measuring test article-induced cytotoxicity.

Accordingly, a cellular viability assay was conducted to assess the impact of **AC ExoEternol** on cellular homeostasis in cultured dermal fibroblasts. Additionally, retinol and bakuchiol were assessed to demonstrate the superior nature of **AC ExoEternol**.

Assay Principle

Cells are incubated with test articles and cellular viability is assessed. This assay utilizes a nonfluorescent dye, resazurin, which is converted to a fluorescent dye, resorufin, in response to chemical reduction by the tricarboxylic acid cycle. Healthy cells easily convert resazurin into resorufin without harming the cells.

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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
C. Equipment:	Forma humidified incubator; ESCO biosafety laminar flow hood; Synergy HT Microplate reader; Pipettes; Light microscope
D. Cell Line:	Normal Human Dermal Fibroblasts (ATCC; PCS-201-012)*
E. Media/Buffers:	Fibroblast Basal Medium (ATCC; PCS-201-030)*; Fibroblast Growth Kit (ATCC; PCS-201-041)*
F. Tissue Culture Plates:	Falcon flat bottom 96-well tissue culture treated plates*
G. Software:	Excel Analysis ToolPak (Microsoft)
H. Reagents:	PrestoBlue™ Reagent (10X)*
I. 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 serial dilution was performed resulting in **AC ExoEternol** concentrations of 0.01%, 0.05%, 0.1%, and 1.0% in complete serum-free media. Additionally, 10-fold serial dilutions of retinol and bakuchiol were prepared in complete serum-free media. Fibroblasts were incubated with designated conditions for 24 hours.

The assay was performed according to the manufacturer's instructions. Briefly, test media was removed and replaced with 10 µL of viability reagent diluted in 90 µL of Complete Media. Cells were incubated with the viability reagent for 2 hours, then fluorometric measurements were taken at 560 nm for excitation and 590 nm for emission.

Experiments were repeated three separate times with each sample run in duplicate. Duplicates for each replicate were averaged, and the average of all three experiments is displayed. Data was analyzed using a one-way ANOVA with statistical significance accepted at $p \leq 0.05$. Cellular viability results are shown as mean fluorescence units (MFU) and expressed as percentage change, calculated by the following equation:

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

Results

The data obtained met criteria for a valid assay and the control performed as anticipated. Compared to Complete Media (untreated fibroblasts), retinol and bakuchiol exhibited negative effects on cellular viability at most concentrations tested. Fibroblasts treated with **AC ExoEternol** did not exhibit negative effects on cellular viability compared to Complete Media.

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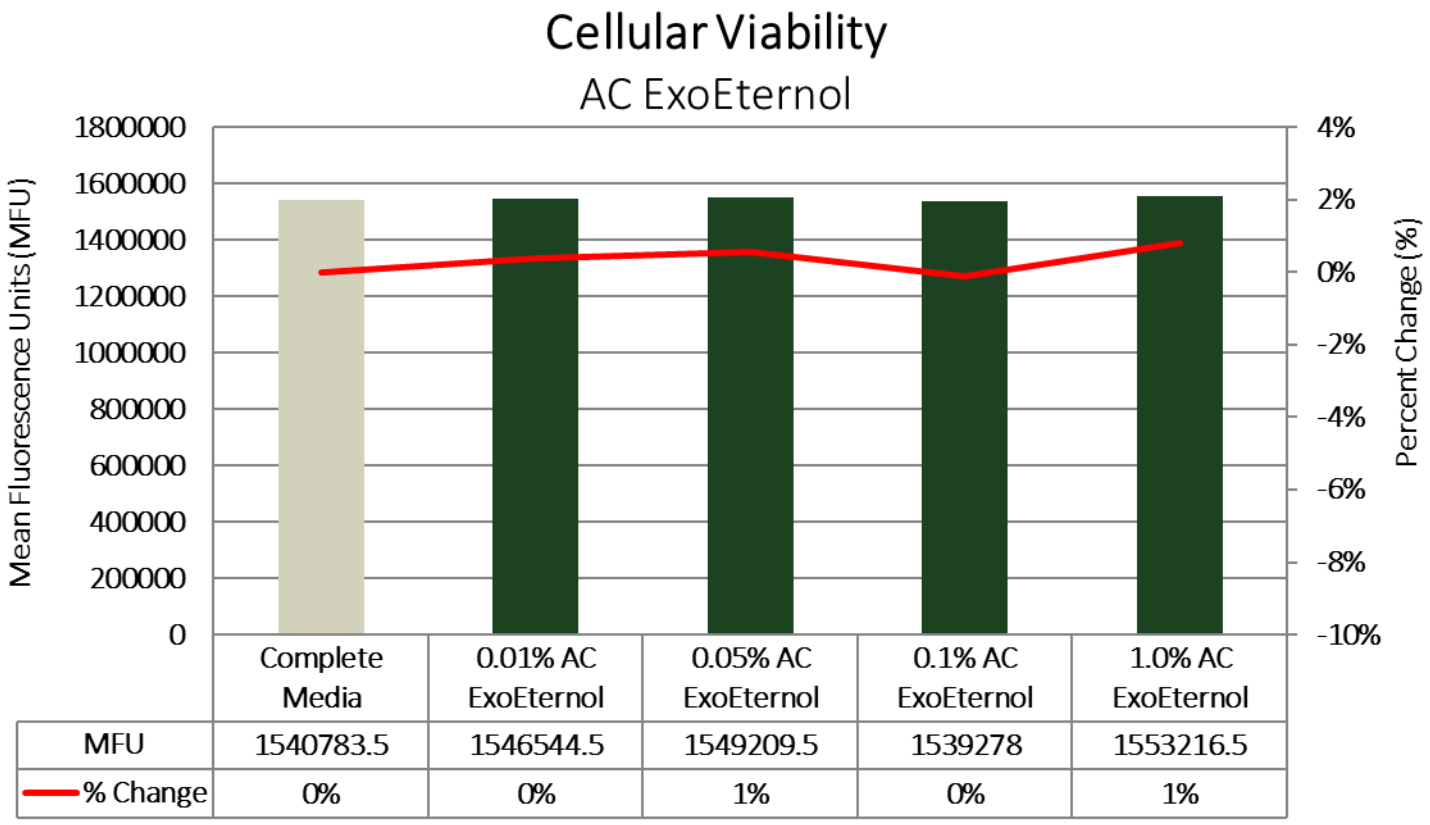

Figure 1. Cellular Viability of Fibroblasts Treated with **AC ExoEternol**. * indicates significance ($p < 0.05$) compared to Complete Media.

Table 1. Results from one-way ANOVA Statistical Analysis of **AC ExoEternol** Compared to Complete Media. * indicates significance ($p < 0.05$) compared to Complete Media.

	0.01% **AC ExoEternol**	0.05% **AC ExoEternol**	0.1% **AC ExoEternol**	1.0% **AC ExoEternol**
P-value	> 0.05	> 0.05	> 0.05	> 0.05

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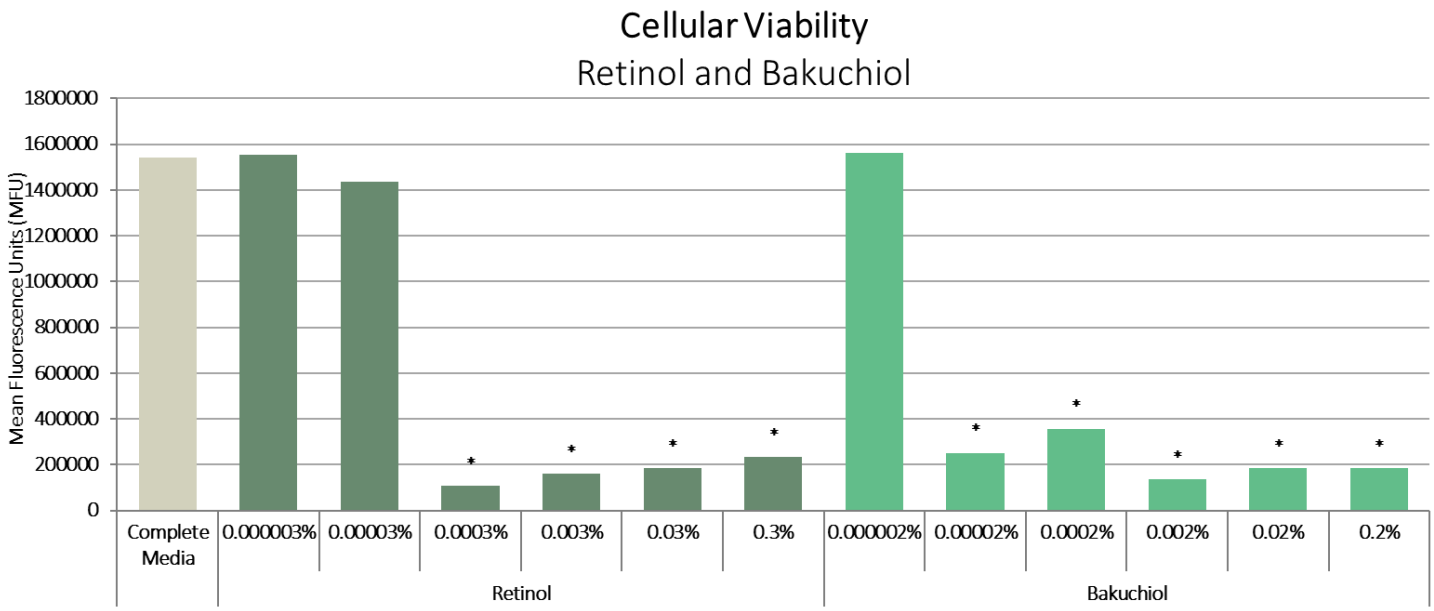

Figure 2. Cellular Viability of Fibroblasts Treated with retinol and bakuchiol. * indicates significance ($p < 0.05$) compared to Complete Media.

Table 2. Results from one-way ANOVA Statistical Analysis of retinol Compared to Complete Media. * indicates significance ($p < 0.05$) compared to Complete Media.

	0.000003%	0.00003%	0.0003%	0.003%	0.03%	0.3%
P-value	> 0.05	> 0.05	< 0.001*	< 0.001*	< 0.001*	< 0.001*

Table 3. Results from one-way ANOVA Statistical Analysis of bakuchiol Compared to Complete Media. * indicates significance ($p < 0.05$) compared to Complete Media.

	0.000002%	0.00002%	0.0002%	0.002%	0.02%	0.2%
P-value	> 0.05	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*

Discussion

In this study, retinol, bakuchiol, and **AC ExoEternol** were tested to evaluate their effects on cellular viability. Dermal fibroblasts exposed to concentrations of 0.0003% and higher of retinol exhibited significant inhibition in cell viability. Similarly, dermal fibroblasts exposed to concentrations of 0.00002% and higher of bakuchiol exhibited significant inhibition in cell viability.

Alternatively, fibroblasts exposed to 0.01%, 0.05%, 0.1%, and 1.0% concentrations of **AC ExoEternol**, and the preservatives contained therein, did not exhibit any significant inhibition in cell viability. Accordingly, these results demonstrate **AC ExoEternol** is not cytotoxic when utilized at the recommended use levels.