



Cellular Viability Assay Analysis

info@activeconceptsllc.com • Phone: +1-704-276-7100 • Fax: +1-704-276-7101

Tradename: AC OleaShield

Code: 12006

CAS #: 68333-16-4 (or) 92128-79-5 & 8001-25-0 & 68333-16-4 (or) 1686112-36-6 (or) 9015-54-7

Test Request Form #: 8468

Lot #: N210818E

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*

Study Director: *Maureen Danaher*

Principle Investigator: *Daniel Shill*

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 OleaShield** 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.

Information contained in this technical literature is believed to be accurate and is offered in good faith for the benefit of the customer. The company, however, cannot assume any liability or risk involved in the use of its chemical products since the conditions of use are beyond our control. Statements concerning the possible use of our products are not intended as recommendations to use our products in the infringement of any patent. We make no warranty of any kind, expressed or implied, other than that the material conforms to the applicable standard specification.



Cellular Viability Assay Analysis

info@activeconceptsllc.com • Phone: +1-704-276-7100 • Fax: +1-704-276-7101

Materials

- A. Kit:** PrestoBlue™ Cell Viability Reagent (Invitrogen, A13261)
- B. Incubation Conditions:** 37°C at 5% CO₂ 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:** Basal Medium (Fibrolife; LM-0001), 500µg/mL Human Serum Albumins (Fibrolife; LS-1001), 0.6µM Linoleic Acid (Fibrolife; LS-1001), 0.6µg/mL (Fibrolife; LS-1001), 5ng/mL Fibroblast Growth Factor (Fibrolife; LS-1002), 5mg/mL Epidermal Growth Factor (Fibrolife; LS-1003), 30pg/mL Transforming Growth Factor β-1 (Fibrolife; LS-2003), 7.5mM L-Glutamine (Fibrolife; LS-1006), 1µg/mL Hydrocortisone Hemisuccinate (Fibrolife; LS-1007), 50µg/mL Ascorbic Acid (Fibrolife; LS-1005), 5µg/mL Insulin (Fibrolife; LS-1004)
- 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 serum-free media. A 10-fold serial dilution was performed resulting in **AC OleaShield** concentrations of 0.1% and 0.01% in complete serum-free media 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.

Information contained in this technical literature is believed to be accurate and is offered in good faith for the benefit of the customer. The company, however, cannot assume any liability or risk involved in the use of its chemical products since the conditions of use are beyond our control. Statements concerning the possible use of our products are not intended as recommendations to use our products in the infringement of any patent. We make no warranty of any kind, expressed or implied, other than that the material conforms to the applicable standard specification.

Results

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

AC OleaShield did not exhibit negative effects on cell metabolism.

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

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

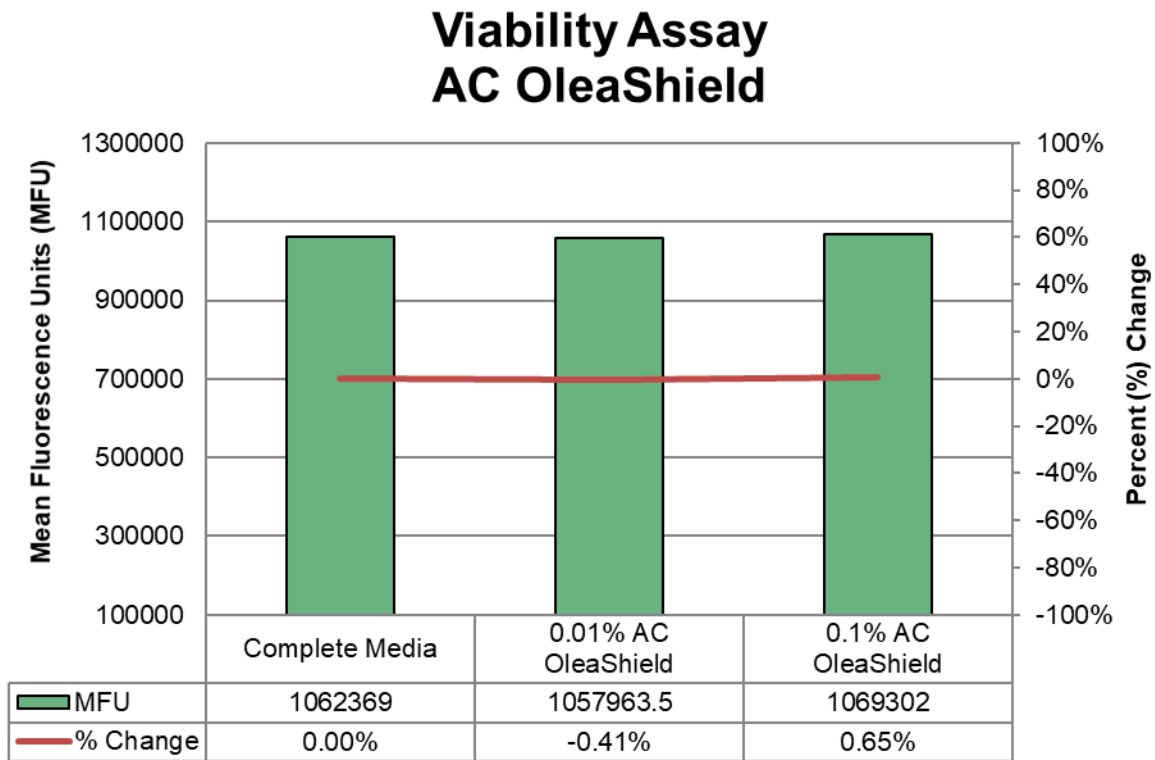


Figure 1: Cellular Metabolism of **AC OleaShield**-treated fibroblasts

Discussion

In this study, **AC OleaShield (12006)** was tested to evaluate its effects on the viability of normal human dermal fibroblasts (NDHF). At concentrations of 0.1% and 0.01%, **AC OleaShield**, nor the preservatives contained therein exhibited any inhibition of cell viability. It can therefore be concluded that at normal use concentrations **AC OleaShield** is not cytotoxic.

Information contained in this technical literature is believed to be accurate and is offered in good faith for the benefit of the customer. The company, however, cannot assume any liability or risk involved in the use of its chemical products since the conditions of use are beyond our control. Statements concerning the possible use of our products are not intended as recommendations to use our products in the infringement of any patent. We make no warranty of any kind, expressed or implied, other than that the material conforms to the applicable standard specification.