Cellular Viability Assay Analysis

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Tradename: Leucidal® SF Max Powder

Code: M15031

CAS #: 1686112-36-6 (or) 68333-16-4

Test Request Form #: 10258

Lot #: 9394433

Sponsor: *Active Micro Technologies, 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 **Leucidal® SF Max Powder** 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:	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 **Leucidal® SF Max Powder** concentrations of 0.01%, and 0.1% 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.

Results

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

Leucidal® SF Max Powder 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:

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

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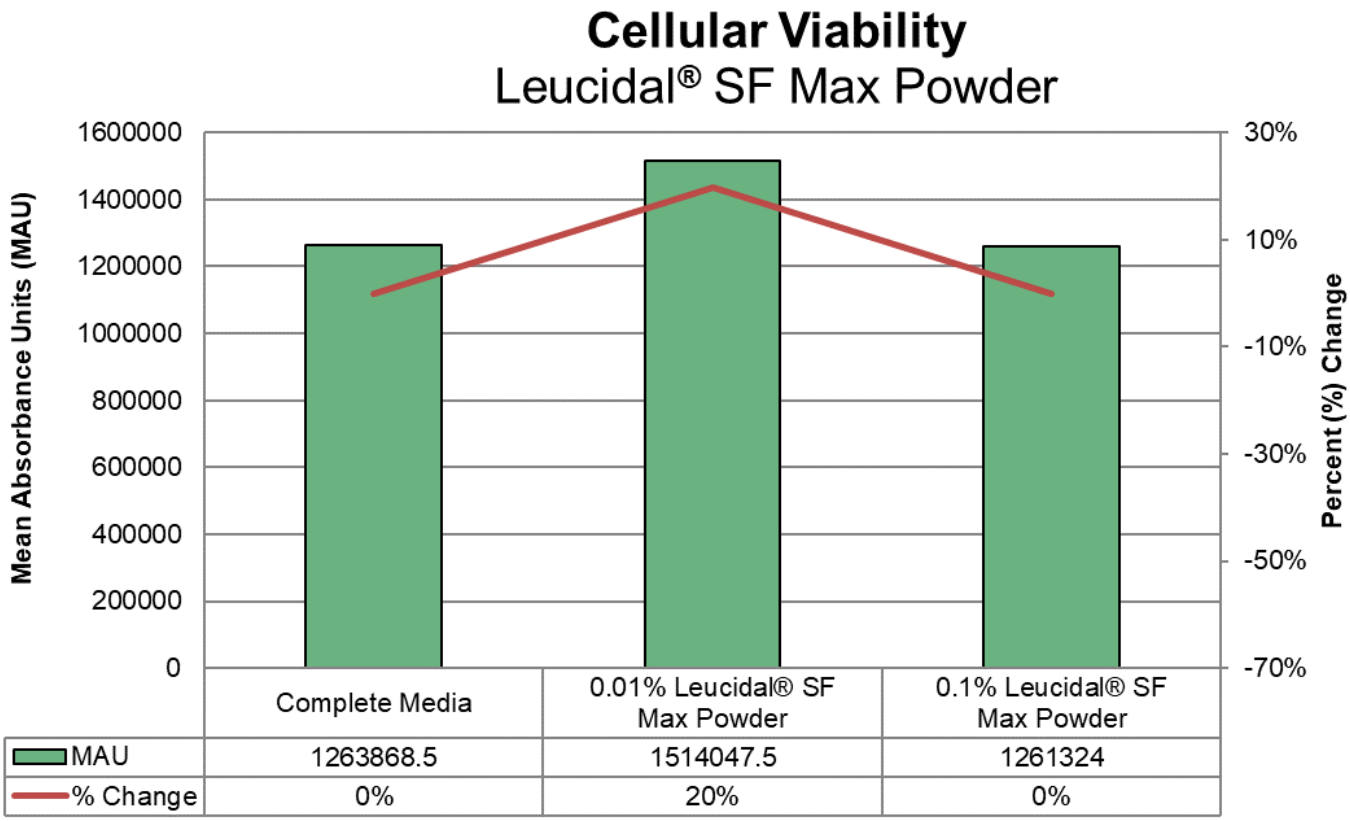

Figure 1: Cellular Metabolism of **Leucidal® SF Max Powder-**Treated Fibroblasts

Discussion

In this study, **Leucidal® SF Max Powder** (**M15031**) was tested to evaluate its effects on the viability of normal human dermal fibroblasts (NDHF). At concentrations of 0.01%, and 0.1%, **Leucidal® SF Max Powder**, nor the preservatives contained therein exhibited any inhibition of cell viability. It can therefore be concluded that at normal use concentrations **Leucidal® SF Max Powder** is not cytotoxic.

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