MTT Cell Viability Assay Analysis

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Tradename: PhytoCycle® Orange

Code: 16925

CAS #: 7732-18-5 & 84012-28-2 (or) 8028-48-6 & 1686112-36-6 (or) 68333-16-4

Test Request Form #: 7093

Lot #: N200818K

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Michael Hovis*

Test Performed:
MTT Cell Viability Assay

Introduction

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) Cell Proliferation assay is useful for determining cell number using a microplate absorbance reader. Determination of cell growth rates is widely used in the testing of drug action, cytotoxic agents, and screening other biologically active compounds. A decrease in MTT turnover indicates cytotoxicity whereas an increase in MTT turnover indicates healthy cells.

The MTT Cell Proliferation assay was conducted to assess the ability of **PhytoCycle® Orange** to increase cellular metabolic activity in cultured dermal fibroblasts.

Assay Principle

The assay is a colorimetric assessment of cellular metabolism. It involves the conversion of water soluble MTT into insoluble formazan. The formazan is then solubilized with sodium dodecyl sulfate (SDS), and the concentration is determined using a microplate reader. A higher concentration of solubilized formazan indicates a higher cellular viability.

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Materials

A. Kit:	Vybrant® MTT Cell Proliferation Assay Kit (Invitrogen, V-13154)*
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 (ATCC; PCS-201-012)*
E. Media/Buffers:	Fibroblast Basal Medium (ATCC; PCS-201-030)*; Fibroblast Growth Kit (ATCC; PCS-201-041)*; Phosphate Buffered Saline (PBS)
F. Reagents:	MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Invitrogen, V-13154)*; Sodium Dodecyl Sulfate (SDS) (Invitrogen, V-13154)*; HCl (0.01 M) (Invitrogen, V-13154)*
G. Culture Plate:	Flat Bottom 96 Well Tissue Culture Treated Microplates
H. 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 media (CM). A 10-fold serial dilution was performed resulting in **PhytoCycle® Orange** concentrations of 0.01% and 0.1% in CM and incubated with fibroblasts for 24 hours. An internal control of just CM and no cells was utilized. All conditions were tested in duplicate.

CM with and without the test substance was removed and replaced with 100 µL of fresh CM. MTT was added to each well and the culture plate was incubated at 37°C for 4 hours. 100 µL of the SDS-HCl solution was added to each well and the culture plate was incubated for an additional 4 hours at 37°C. Following this incubation, absorbance was measured at 570 nm.

Cellular viability results are shown as mean absorbance units (MAU) and expressed as percentage change, as calculated by the following equation:

$$Percent\ Change\ (\%) = \frac{MAU_{CM} - MAU_{Sample}}{MAU_{CM}} \times 100$$

Results

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

PhytoCycle® Orange did not exhibit negative effects on cell viability.

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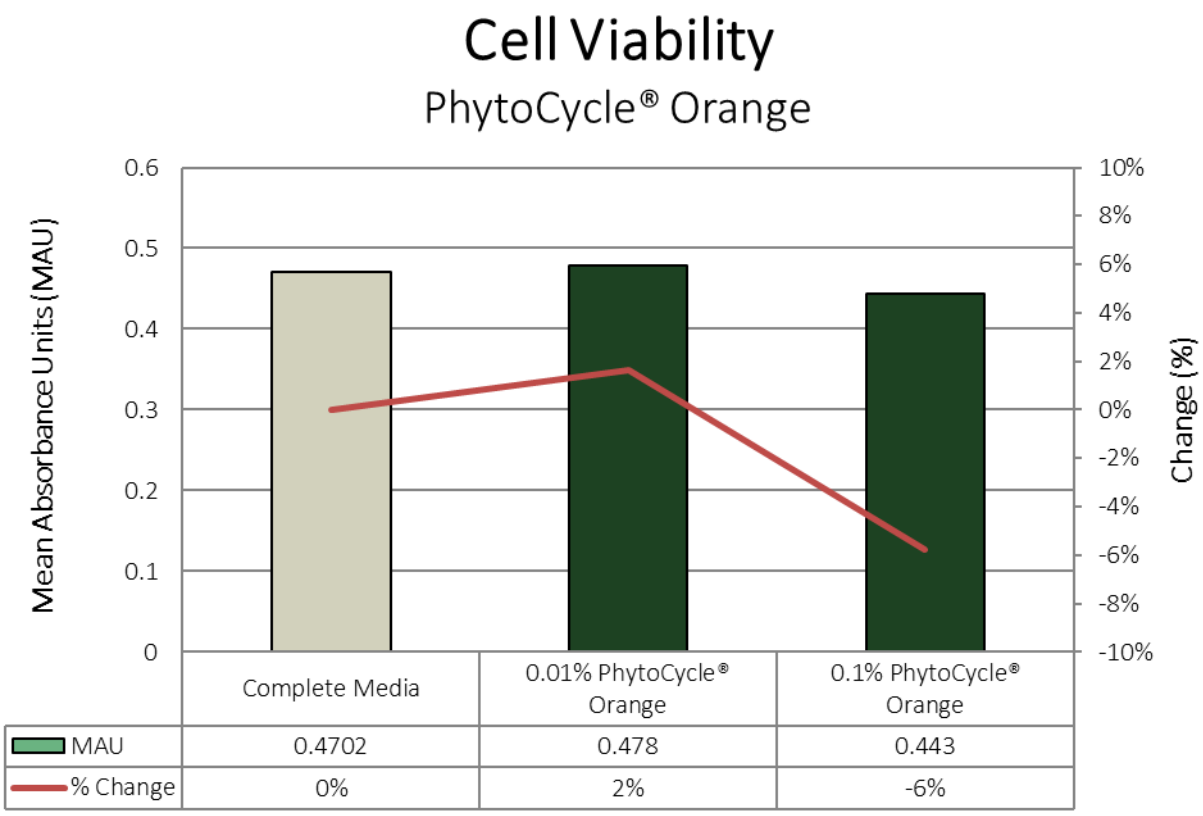

Figure 1. The effect of **PhytoCycle® Orange** on fibroblast cellular viability.

Discussion

PhytoCycle® Orange was tested to evaluate its effects on the viability of normal human dermal fibroblasts. Fibroblasts treated with 0.01% and 0.1% concentrations of **PhytoCycle® Orange** did not exhibit any reductions in cellular viability. Accordingly, at normal use concentrations, **PhytoCycle® Orange** is not cytotoxic.