Active Concepts

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Cellular Viability Assay Analysis

Tradename: AC Plant Keratin PF

Code: 20624PF

CAS #: 100209-41-4 & 70084-87-6 (or) 94350-06-8 & 68607-88-5

Test Request Form #: 465

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

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 is 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 Plant Keratin PF** 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:	Cellular Senescence Assay Kit (Chemicon® International; KAA002)
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:	Dulbecco's Modified Eagle Medium (DMEM); Penicillin-Streptomycin (50U-50mg/mL); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS)
F. Culture Plate:	Falcon flat bottom 6-well tissue culture treated plates
G. Reagents:	5-bromo-4-chloro-indolyl-β-D-galactopyranoside (X-gal)
H. Other:	Sterile disposable pipette tips; wash bottles; 15mL conical tubes, 1.5mL microcentrifuge tubes

Methods

Human dermal fibroblasts were seeded into 96-well tissue culture plates and allowed to grow to confluency in complete DMEM. A 10-fold serial dilution was performed resulting in **AC Plant Keratin PF** concentrations on 1%, 0.1%, and 0.01% in complete DMEM and incubated with fibroblasts for 24 hours.

Ten microliters of viability reagent was added to 90µL of cell culture media in culture wells.

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Results

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

AC Plant Keratin PF exhibited positive effects on cellular metabolism compared to the control.

Cellular metabolism results are expressed as a percentage of the control.

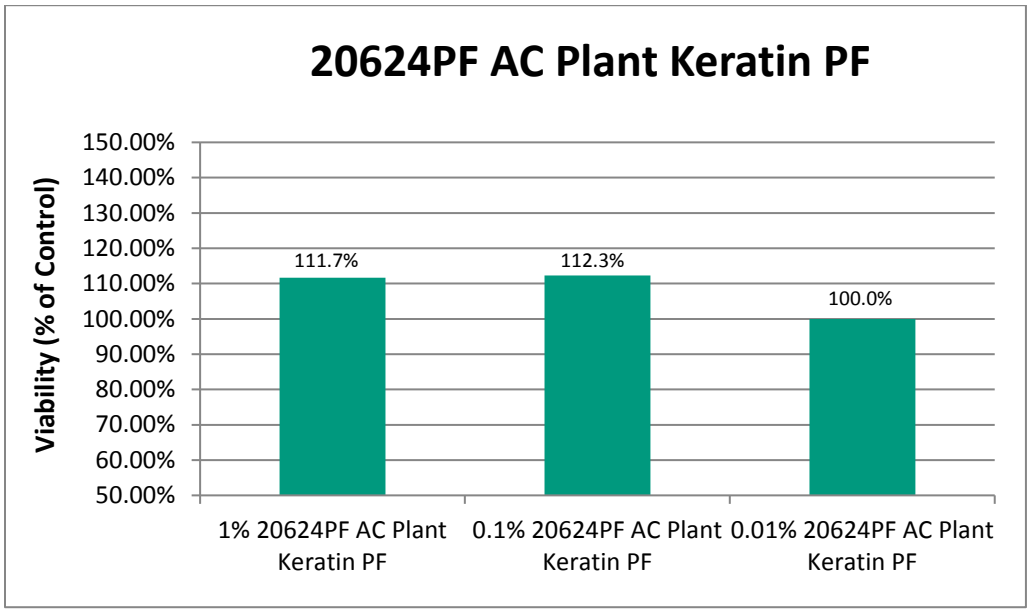

Figure 1: Cellular Metabolism of **AC Plant Keratin PF** -treated fibroblasts expressed in terms of percent of control.

Discussion

As shown in figure 1, **AC Plant Keratin PF** exhibited positive results by increasing cell metabolism. The increase in fluorescent signal indicates an increase in cellular metabolism and viability post **AC Plant Keratin PF** treatment. For these reasons, we can assume **AC Plant Keratin PF** is suitable for cosmetic applications designed to increase cell viability and metabolism.