

Cellular Viability Assay Analysis

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Tradename: ACB Quinoa Protein

Code: 20037

CAS #: 100209-45-8

Test Request Form #: 1270

Lot Number: NC-150122-C

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 **ACB Quinoa Protein** 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% 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: 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 **ACB Quinoa Protein** 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.

ACB Quinoa Protein exhibited significant effects on cellular metabolism compared to the control.

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

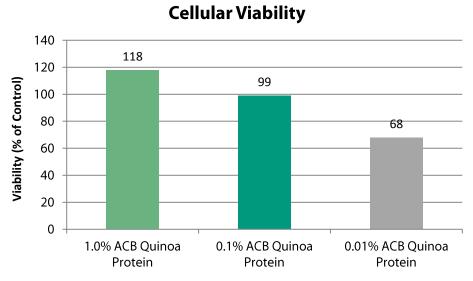


Figure 1: Cellular Metabolism of **ACB Quinoa Protein**-treated fibroblasts expressed in terms of percent of control.

Discussion

As shown in figure 1, **ACB Quinoa Protein** exhibited comparable results by increasing cell metabolism. An increase in fluorescent signal indicates an increase in cellular metabolism and viability. **ACB Quinoa Protein** does not appear to have negative effects on cellular metabolism and can safely be used in cosmetic materials.