

Cellular Viability Assay Analysis

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

<u>Tradename:</u> ACB Yogurt Extract

Code: 20070

CAS #: 7732-18-5 & N/A

Test Request Form #: 372

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

Study Director: Erica Segura

Principle Investigator: Meghan Darley

<u>Test Performed:</u> 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 Yogurt Extract** 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% 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 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 DMEM. A 10-fold serial dilution was performed resulting in **ACB Yogurt Extract** 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 Yogurt Extract exhibited positive effects on cellular metabolism.

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

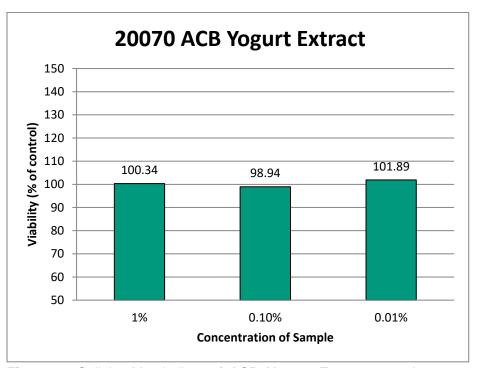


Figure 1: Cellular Metabolism of **ACB Yogurt Extract**-treated fibroblasts expressed in terms of percent of control.

Discussion

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

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