



# Cellular Viability Assay Analysis

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**Tradename:** ACB Yogurt Dermal Respiratory Factor PF

**Code:** 20224PF

**CAS #:** 68333-15-3

**Test Request Form #:** 937

**Lot #:** 36751

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

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**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 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 **ACB Yogurt Dermal Respiratory Factor 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:** PrestoBlue™ Cell Viability Reagent (Invitrogen, A13261)
- B. **Incubation Conditions:** 37°C at 5% CO<sub>2</sub> 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 Dermal Respiratory Factor 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 and a fluorometric measurement was taken at 560nm for excitation and 590nm for emission.

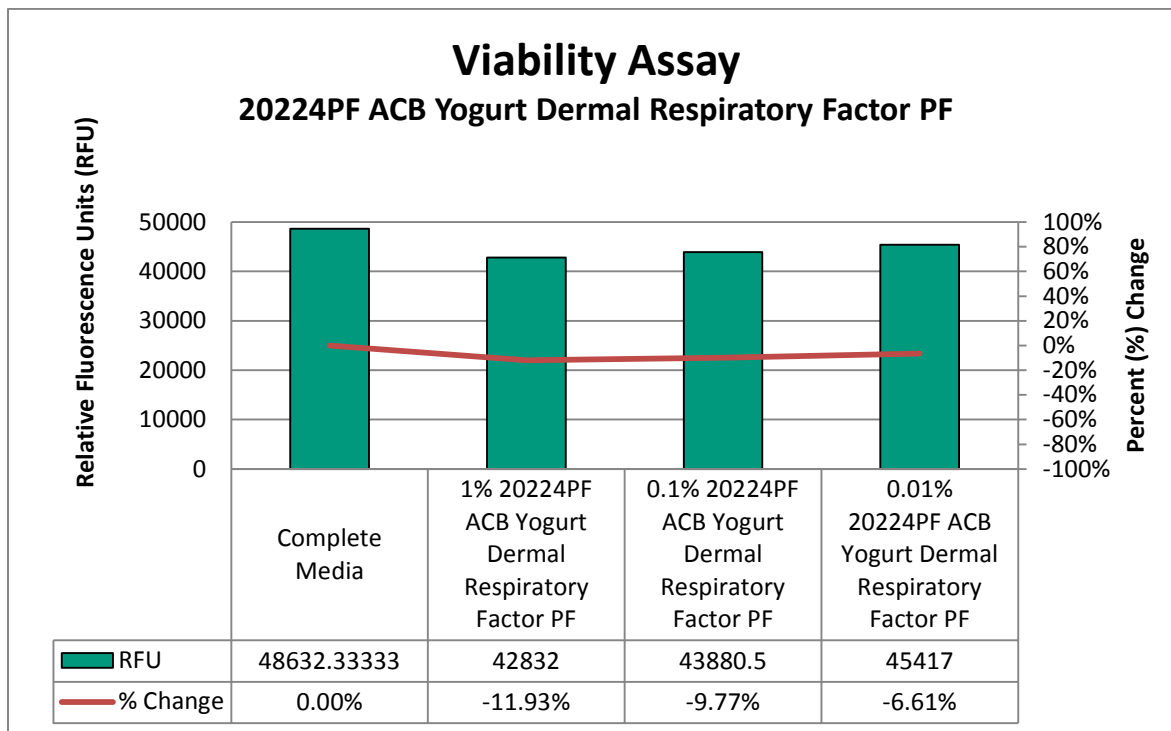
## Results

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

**ACB Yogurt Dermal Respiratory Factor PF** did not have 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:

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



**Figure 1:** Cellular Metabolism of **ACB Yogurt Dermal Respiratory Factor PF**-treated fibroblasts expressed in terms of percent of control.

## Discussion

In this study, **ACB Yogurt Dermal Respiratory Factor PF** (code 20224PF) was tested to evaluate its effects on the viability of normal human dermal fibroblasts (NDHF). At concentrations of 1%, 0.1%, and 0.01% **ACB Yogurt Dermal Respiratory Factor PF** (code 20224PF), nor the preservatives contained therein exhibited any inhibition of cell viability. It can therefore be concluded that at normal use concentrations **ACB Yogurt Dermal Respiratory Factor PF** (code 20224PF) is not cytotoxic.

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