



## Cellular Viability Assay Analysis

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**Tradename:** AC Rice Curl Complex PF

**Code:** 20650PF

**CAS #:** 7732-18-5 & 90131-63-8 & 68553-81-1 & 65072-01-7 & 85-61-0

**Test Request Form #:** 466

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

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**Principle Investigator:** *Maureen Danaher*

**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 **AC Rice Curl Complex 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

- |                                  |   |
|----------------------------------|---|
| <b>A. Kit:</b>                   | PrestoBlue™ Cell Viability Reagent (Invitrogen, A13261)   |
| <b>B. Incubation Conditions:</b> | 37°C at 5% CO <sub>2</sub> and 95% relative humidity (RH)   |
| <b>C. Equipment:</b>             | Forma humidified incubator; ESCO biosafety laminar flow hood; Light microscope; Pipettes  |
| <b>D. Cell Line:</b>             | Normal Human Dermal Fibroblasts (NHDF) (Lonza; CC-2511)   |
| <b>E. Media/Buffers:</b>         | Dulbecco's Modified Eagle Medium (DMEM); Penicillin-Streptomycin (50U-50mg/mL); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS) |
| <b>F. Culture Plate:</b>         | Falcon flat bottom 96-well tissue culture treated plates  |
| <b>G. Reagents:</b>              | PrestoBlue™ reagent (10X)   |
| <b>H. Other:</b>                 | 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 **AC Rice Curl Complex PF** concentrations on 1%, 0.1%, and 0.01% in complete DMEM and incubated with fibroblasts for 24 hours.

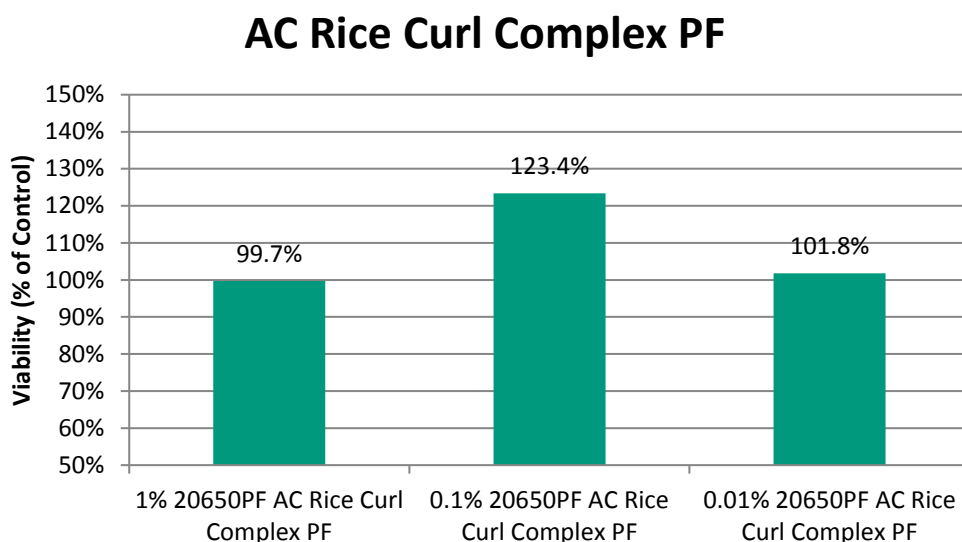
Ten microliters of viability reagent was added to 90µL of cell culture media in culture wells.

## Results

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

**AC Rice Curl Complex 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 Rice Curl Complex PF**-treated fibroblasts expressed in terms of percent of control.

## Discussion

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