



## Cellular Viability Assay Analysis

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**Tradename:** Revital-Eyes

**Code:** 16671

**CAS #:** 7732-18-5 & 68333-16-4 & 84650-60-2 & 84961-57-9 & 1686112-36-6 (or)  
68333-16-4 & 58-08-2

**Test Request Form #:** 7286

**Lot #:** 76980P

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

**Study Director:** *Maureen Danaher*

**Principle Investigator:** *Michael Hovis*

**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 **Revital-Eyes** 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:** Basal Medium (Fibrolife; LM-0001), 500µg/mL Human Serum Albumins (Fibrolife; LS-1001), 0.6µM Linoleic Acid (Fibrolife; LS-1001), 0.6µg/mL (Fibrolife; LS-1001), 5ng/mL Fibroblast Growth Factor (Fibrolife; LS-1002), 5mg/mL Epidermal Growth Factor (Fibrolife; LS-1003), 30pg/mL Transforming Growth Factor β-1 (Fibrolife; LS-2003), 7.5mM L-Glutamine (Fibrolife; LS-1006), 1µg/mL Hydrocortisone Hemisuccinate (Fibrolife; LS-1007), 50µg/mL Ascorbic Acid (Fibrolife; LS-1005), 5µg/mL Insulin (Fibrolife; LS-1004)
- 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 serum-free media. A 10-fold serial dilution was performed resulting in **Revital-Eyes** concentrations of 1.0%, 0.1%, and 0.01% in complete serum-free media 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.

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## Results

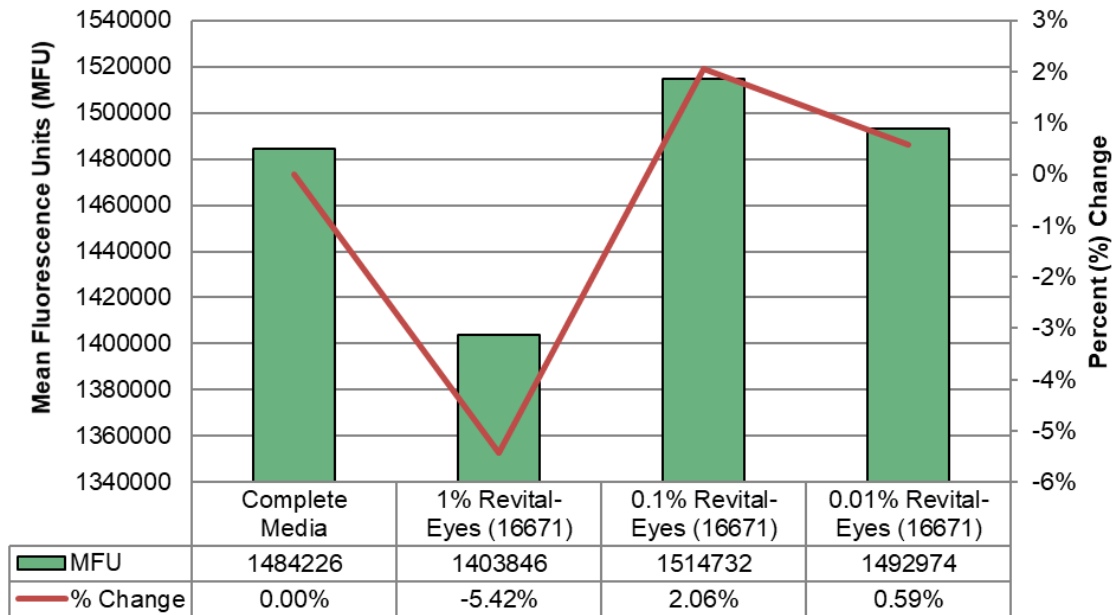
The data obtained from this study met criteria for a valid assay and the controls performed as anticipated.

**Revital-Eyes** did not exhibit 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:

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

### Viability Assay Revital-Eyes



**Figure 1:** Cellular Metabolism of **Revital-Eyes**-treated fibroblasts

## Discussion

In this study, **Revital-Eyes** (16671) was tested to evaluate its effects on the viability of normal human dermal fibroblasts (NDHF). At concentrations of 0.1% and 0.01%, **Revital-Eyes**, nor the preservatives contained therein exhibited any inhibition of cell viability. It can therefore be concluded that at normal use concentrations **Revital-Eyes** is not cytotoxic.

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