# MTT Cell Viability Assay Analysis

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**Tradename:** AC Water Kefir PHA

**Code:** 22064

**CAS #:** 7732-18-5 & 90082-21-6 & 1686112-36-6 (or) 68333-16-4 (or) 9015-54-7

**Test Request Form #:** 6991

**Lot #:** N200812H

**Sponsor:** *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
**Study Director:** *Maureen Danaher*
**Principle Investigator:** *Michael Hovis*

**Test Performed:**
MTT Cell Viability Assay

## Introduction

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) Cell Proliferation assay is useful for determining cell number using a microplate absorbance reader. Determination of cell growth rates is widely used in the testing of drug action, cytotoxic agents, and screening other biologically active compounds. A decrease in MTT turnover indicates cytotoxicity whereas an increase in MTT turnover indicates healthy cells.

The MTT Cell Proliferation assay was conducted to assess the ability of **AC Water Kefir PHA** to increase cellular metabolic activity in cultured dermal fibroblasts.

## Assay Principle

The assay is a colormetric assessment of cellular metabolism. It involves the conversion of water soluble MTT into insoluble formazan. The formazan is then solubilized with sodium dodecyl sulfate (SDS), and the concentration is determined using a microplate reader. A higher concentration of solubilized formazan indicates a higher cellular viability.

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## Materials

| | | |
|---|---|---|
| **A.** | **Kit:** | Vybrant® MTT Cell Proliferation Assay Kit (Invitrogen, V-13154) |
| **B.** | **Incubation Conditions:** | 37°C at 5% $CO_2$ and 95% relative humidity (RH) |
| **C.** | **Equipment:** | Forma humidified incubator; ESCO biosafety laminar flow hood; Light microscope; Microplate Reader; Pipettes |
| **D.** | **Cell Line:** | Normal Human Dermal Fibroblasts (HDFa) (ATCC; PCS-201-012) |
| **E.** | **Media/Buffers:** | Dulbecco's Modified Eagle Medium (DMEM); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS) |
| **F.** | **Culture Plate:** | Falcon flat bottom 96-well tissue culture treated plates |
| **G.** | **Reagents:** | MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); Sodium Dodecyl Sulfate (SDS); HCl (0.01 M) |
| **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 media. A 10-fold serial dilution was performed resulting in **AC Water Kefir PHA** concentrations of 0.1% and 0.01% in complete media and incubated with fibroblasts for 24 hours.

Media was removed and replaced with 100µL of fresh complete media. Ten microliters of 12 mM MTT stock solution was added to each well. A negative control of 10 µL MTT stock solution added to 100 µL fresh complete media was used. The tissue culture plate was incubated at 37°C for 4 hours. 100 µL of the SDS-HCl solution was added to each well and thoroughly mixed. The tissue culture plate was incubated for an additional 4 hours at 37°C. After the incubation, each well was mixed well, and an absorbance measurement was taken at 570nm.

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## Results

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

**AC Water Kefir PHA** did not exhibit negative effects on cell viability.

Cellular viability results are shown as mean absorbance units (MAU) and expressed as percentage change, calculated by the below equation:

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

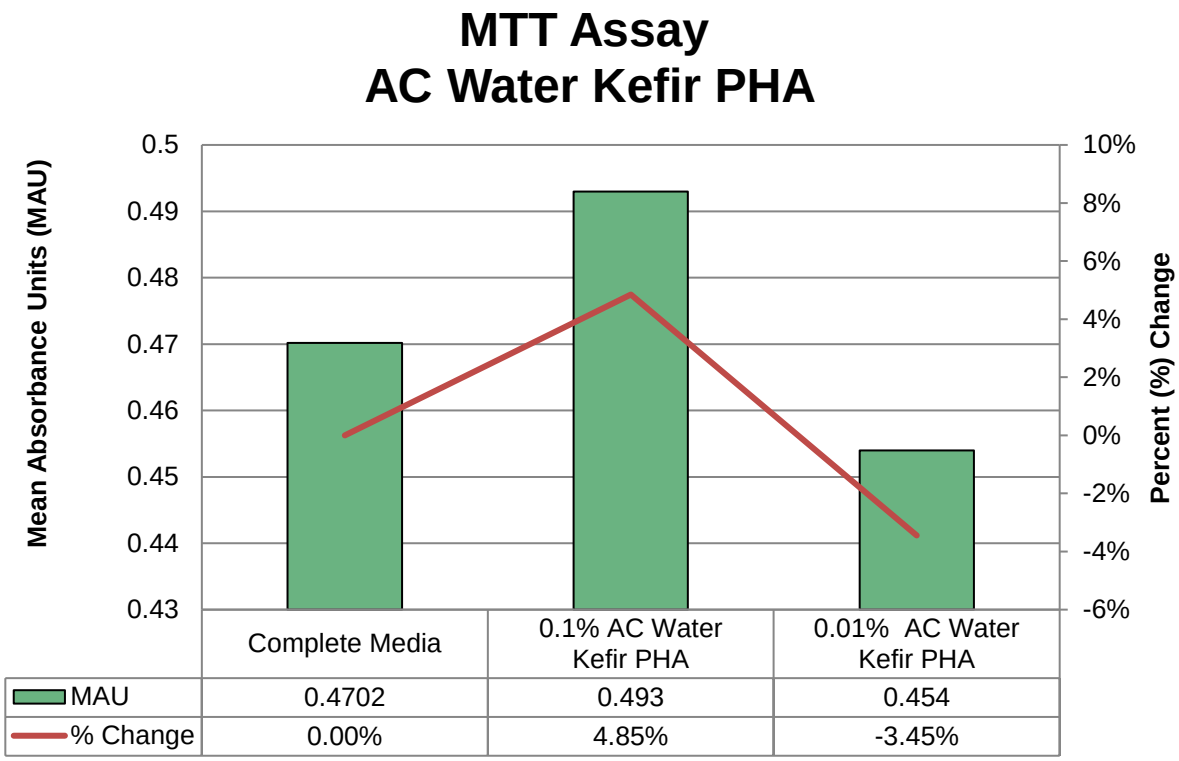


**Figure 1:** Cellular Viability of **AC Water Kefir PHA** - treated fibroblasts

## Discussion

In this study, **AC Water Kefir PHA** (22064) was tested to evaluate its effects on the viability of normal human dermal fibroblasts. At concentrations of 0.1% and 0.01%, **AC Water Kefir PHA**, nor the preservatives contained therein exhibited any inhibition of cell viability. It can therefore be concluded that at normal use concentrations **AC Water Kefir PHA** is not cytotoxic.