

**Tradename:** AC LumiVitis

**Code:** 21032

**CAS #:** 8013-01-2 & 85594-37-2 (or) 84929-27-1 & 68333-16-4 (or) 1686112-36-6 (or) 9015-54-7

**Test Request Form #:** 13647

**Lot #:** 9418748

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

**Study Director:** *Daniel Shill*

**Principal Investigator:** *Hannah Stade*

**Test Performed:**

Matrix Metalloproteinase (MMP)-1 Enzyme-Linked Immunosorbent Assay (ELISA)

**Introduction**

Matrix metalloproteinase-1 (MMP-1) is a key enzyme responsible for remodeling and degrading the extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling. MMP-1 is activated as cells age but can be exacerbated by UV-B light or blue light irradiation. When activated, MMP-1 breaks down interstitial collagen leading to wrinkles, fine lines, and loss of skin elasticity. Reducing MMP-1 activity will therefore decrease collagen degradation and promote a more youthful skin appearance.

Accordingly, a matrix metalloproteinase-1 ELISA was conducted to assess the *in vitro* effect of **AC LumiVitis** to elicit changes in MMP-1 levels in dermal fibroblasts.

**Assay Principle**

This ELISA utilizes a colorimetric reaction employing antibodies with antigen specificity to human MMP-1. Antibodies specific for MMP-1 epitopes are coated on a microtiter plate. In positive samples, MMP-1 will bind to these antibodies and are tagged a second time with another MMP-1-specific antibody labeled with horseradish peroxidase (HRP). The addition of the chromagen solution, containing 3,3',5,5'-tetramethylbenzidine, provides the colorimetric reaction with HRP that is quantitated through optical density (OD) readings on a microplate spectrometer. The standard curve provides a reference from the OD readings for the amount of MMP-1 in each sample.

## Materials

- A. Kit:** Human MMP-1 ELISA Kit (Invitrogen; EHMMP1)\*
- B. Incubation Conditions:** 37°C, 5% CO<sub>2</sub>, and 95% relative humidity (RH)
- C. Equipment:** Forma humidified incubator; ESCO biosafety laminar flow hood; Synergy HT Microplate reader; Pipettes; Light microscope; Accuris UV Transilluminator; elixa LED Blue Light Array
- D. Cell Line:** Normal Human Dermal Fibroblasts (ATCC; PCS-201-012)\*
- E. Media/Buffers:** Fibroblast Basal Medium (PCS-201-030)\*; Fibroblast Growth Kit (PCS-201-041)\*; Phosphate Buffered Saline (PBS)
- F. Reagents:** Resveratrol; DMSO
- G. Software:** Excel Analysis ToolPak (Microsoft)
- H. Culture Plate:** 24 Well Tissue Culture Treated Plates
- I. Other:** Sterile disposable pipette tips

\*Or suitable alternatives, subject to change without notice based off vendor availability

## Methods

Human dermal fibroblasts were seeded into 24-well tissue culture plates and allowed to grow to confluency in complete media (CM). Next, the fibroblasts were irradiated with either UV-B Light or Blue Light at the dosages outlined in Table 1. Designated wells on each plate were incubated with CM as Untreated controls with and without light irradiation. After irradiation, 0.01%, 0.02%, and 0.05% concentrations of **AC LumiVitis** were added to CM and incubated at 37°C with fibroblasts. CM was used at the untreated control, whereas resveratrol (40 µM) was diluted in CM and used as a positive control. Following a 24-hour incubation, the media in all wells collected and stored at -80°C. The media samples were thawed and utilized in the MMP-1 ELISA Kit according to the manufacturer's instructions.

**Table 1.** Light Wavelengths and Dosages

Light Source	Wavelength (nm)	Dose (J/cm <sup>2</sup> )
UV-B Light	302	0.076
Blue Light	470	480

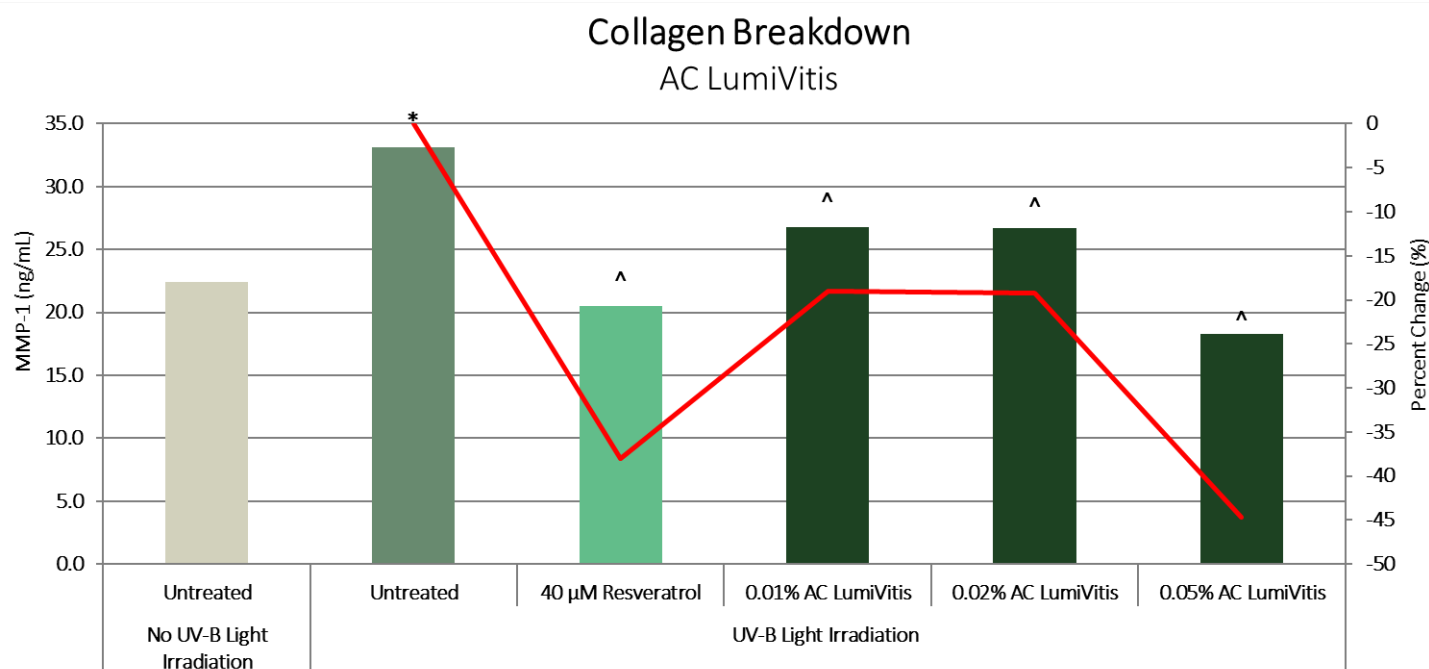
Briefly, standards were prepared in concentrations ranging from 0 pg/mL to 18000 pg/mL. 100 µL of the standards and samples were added to appropriate wells and incubated for two and a half hours. Next, all wells were washed and 100 µL of biotin-conjugate was added to each well. After a one-hour incubation at room temperature and washing, 100 µL Streptavidin-HRP was added to all wells. Following a 45-minute incubation and washing, 100 µL of TMB substrate solution was added for the colorimetric reaction. After a 10-minute incubation in the dark, 50 µL of stop solution was added to stop the reaction. The optical density (OD) was read at 450 nm on the Synergy HT Microplate Reader.

Assays were repeated three separate times for each light source with each sample run in duplicate. Duplicates for each replicate were averaged, and the average of all three experiments is displayed. Data was analyzed using a one-way ANOVA with statistical significance accepted at  $p \leq 0.05$ . A standard curve was created by reducing the data and generating a curve fit. The MMP-1 concentrations were determined by extrapolation from the standard curve and are expressed in ng/mL. Percent change is expressed relative to the untreated control and calculated by the following equation:

$$\text{Percent Change (\%)} = \frac{\text{MMP 1 Concentration}_{\text{Sample}} - \text{MMP 1 Concentration}_{\text{Untreated}}}{\text{MMP 1 Concentration}_{\text{Untreated}}} \times 100$$

## Results

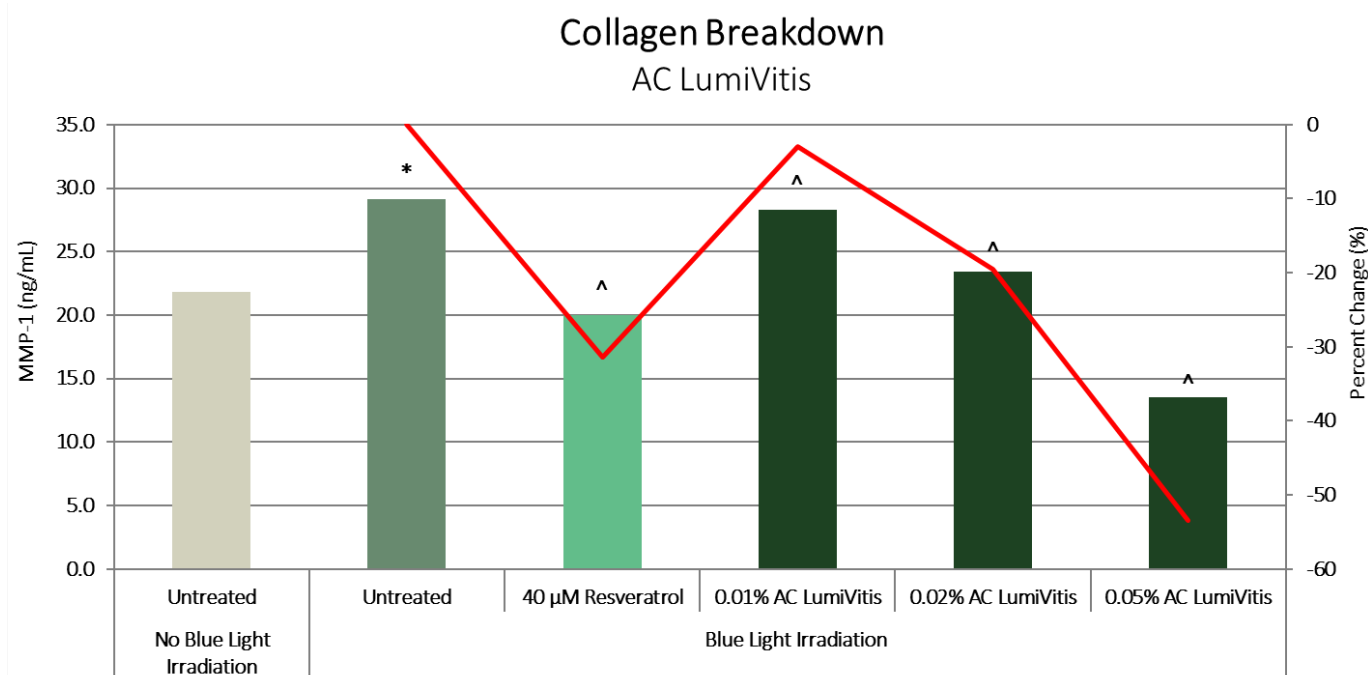
The data obtained met criteria for a valid assay and the controls performed as anticipated. UV-B Light and Blue Light irradiation elicited increased MMP-1 production whereas Resveratrol (40  $\mu$ M) reduced the light-induced collagen degradation. Fibroblasts treated with **AC LumiVitis** demonstrated reductions in light-induced MMP-1 levels.



**Figure 1.** The effect of UV-B Light Irradiation on MMP-1 Concentrations in Fibroblasts. \* indicates significance ( $p \leq 0.05$ ) compared to untreated fibroblasts. ^ indicates significance ( $p \leq 0.05$ ) compared to untreated fibroblasts with irradiation.

**Table 2.** Results from one-way ANOVA Statistical Analysis of MMP-1 levels after UV-B Light Irradiation Compared to Untreated Fibroblasts with and without Irradiation. \* indicates significance ( $p \leq 0.05$ ) compared to untreated fibroblasts. ^ indicates significance ( $p \leq 0.05$ ) compared to untreated fibroblasts with irradiation.

	Untreated + Irradiated	Resveratrol (40 $\mu$ M)	0.01% AC LumiVitis	0.02% AC LumiVitis	0.05% AC LumiVitis
Untreated	0.011*	> 0.05	> 0.05	> 0.05	> 0.05
Untreated + Irradiated	-----	0.023^	0.019^	0.035^	0.016^



**Figure 2.** The effect of Blue Light Irradiation on MMP-1 Concentrations in Fibroblasts. \* indicates significance ( $p \leq 0.05$ ) compared to untreated fibroblasts. ^ indicates significance ( $p \leq 0.05$ ) compared to untreated fibroblasts with irradiation.

**Table 3.** Results from one-way ANOVA Statistical Analysis of MMP-1 levels after Blue Light Irradiation Compared to Untreated Fibroblasts with and without Irradiation. \* indicates significance ( $p \leq 0.05$ ) compared to untreated fibroblasts. ^ indicates significance ( $p \leq 0.05$ ) compared to untreated fibroblasts with irradiation.

	Untreated + Irradiated	Resveratrol (40 μM)	0.01% AC LumiVitis	0.02% AC LumiVitis	0.05% AC LumiVitis
Untreated	< 0.001*	> 0.05	> 0.05	> 0.05	> 0.05
Untreated + Irradiated	-----	0.018^	< 0.001^	0.001^	< 0.001^

## Discussion

As shown in Figure 1, UV-B Light elicited a 48% increase in MMP-1 levels compared to untreated fibroblasts. Conversely, fibroblasts irradiated with UV-B Light and treated with Resveratrol elicited a 38% significant reduction in MMP-1 compared to untreated fibroblasts with irradiation (Table 2). Similarly, fibroblasts treated with **AC LumiVitis** at 0.01%, 0.02%, and 0.05% demonstrated 19%, 19%, and 45% reductions in MMP-1 levels compared to untreated fibroblasts with irradiation, respectively. These data demonstrate **AC LumiVitis** reduces collagen breakdown induced by UV-B irradiation.

Similarly, Blue Light elicited a 34% increase in MMP-1 levels compared to untreated fibroblasts (Figure 2). Conversely, fibroblasts exposed to Blue Light and treated with Resveratrol elicited a 31% significant reduction in MMP-1 compared to untreated fibroblasts with irradiation (Table 3). Similarly, fibroblasts treated with **AC LumiVitis** at 0.01%, 0.02%, and 0.05% demonstrated 3%, 20%, and 53% reductions in MMP-1 levels compared to untreated fibroblasts with irradiation, respectively. These data demonstrate **AC LumiVitis** reduces collagen breakdown induced by Blue Light irradiation.

Collectively, a decrease in MMP-1 production indicates reduced collagen breakdown which could diminish the signs of aging and minimize the formation of fine lines and wrinkles. These data indicate **AC LumiVitis** reduces UV-B Light and Blue Light induced MMP-1 production, which may help to attenuate characteristics of cellular aging.