Oxidative Phosphorylation Assay

ACTIVE CONCEPTS LLC
107 Technology Drive, Lincolnton | NC 28092 USA
• info@activeconceptsllc.com • Phone: +1-704-276-7100 • Fax: +1-704-276-7101 • https://activeconceptsllc.com/

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 #: 14159

Lot #: 9418748

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Daniel Shill*
Principal Investigator: *Hannah Stade*

Test Performed:
Oxidative Phosphorylation (Mitochondrial Membrane Potential) Assay

Introduction

Cellular metabolism can occur with (aerobic) or without (anaerobic) oxygen to generate ATP (adenosine triphosphate), the end molecular byproduct of metabolism. The stored energy within ATP is harvested and utilized by cells to maintain homeostasis as it is required for cellular migration and proliferation, molecular transportation across membranes, and the biosynthesis of cellular components. Aerobic metabolism, also known as oxidative phosphorylation, occurs within the mitochondria, generates a large amount of ATP, and is the primary source of cellular energy transduction. One driving force of oxidative phosphorylation is the mitochondrial membrane potential (ΔΨm), which represents the transmembrane potential of hydrogen ions. Maintaining ΔΨm is necessary as the proton flux from cytosol to matrix is harnessed to generate ATP. This electrochemical gradient is not only utilized for ATP synthesis but is also an indicator of mitochondrial function and health as prolonged depressions in ΔΨm reduce cell viability. Specifically, unregulated mitochondrial membrane potential produces excessive amounts of oxidative stress which lead to an acceleration of DNA mutation, cellular senescence, advanced glycation end products, protein oxidation, and collagen degradation.

Accordingly, a Mitochondrial Membrane Potential Assay was conducted to assess the *in vitro* effect of **AC LumiVitis** to stimulate oxidative phosphorylation in dermal fibroblasts. Activating this biological process maintains cellular homeostasis and vitality.

Assay Principle

The Mitochondrial Membrane Potential Assay is based on the detection of the ΔΨm in cells. The lipophilic and cationic JC-10 dye selectively enters mitochondria and concentrates in the mitochondrial matrix where it fluoresces red. The JC-10 dye reversibly changes color from green to orange as membrane potentials increase due to shifts in emitted light from 520 nm to 570 nm, expressed as modifications in the JC-10 from monomeric to J-aggregate, respectively. As ΔΨm increases, ATP synthesis also increases, thus ΔΨm can be utilized as a surrogate measurement of oxidative phosphorylation.

ACTIVE CONCEPTS LLC
107 Technology Drive, Lincolnton | NC 28092 USA
• info@activeconceptsllc.com • Phone: +1-704-276-7100 • Fax: +1-704-276-7101 • https://activeconceptsllc.com/

Materials

A. Kit:	JC-10 Mitochondrial Membrane Potential Assay Kit (Abcam; ab112134)*
B. Incubation Conditions:	37°C, 5% CO_2, 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 Neonatal Human Dermal Fibroblasts (ATCC; PCS-201-010)*
E. Media/Buffers:	Fibroblast Basal Medium (PCS-201-030)*; Fibroblast Growth Kit (PCS-201-041)*; Ethanol
F. Reagents:	100x JC-10 Dye Solution (ab112134)*; Assay Buffer A (ab112134)*; Assay Buffer B (ab112134)*; Phosphate Buffered Saline (PBS); Antimycin A (Sigma Aldrich, A8674)*
G. Culture Plate:	96 Well Black Side/Clear Bottom Tissue Culture Treated Microplates
H. Software:	Excel Analysis ToolPack (Microsoft)
I. Other:	Sterile disposable pipette tips

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

Methods

Human neonatal dermal fibroblasts were seeded into a 96-well tissue culture microplate and grown to confluency in Complete Media (CM). 0.01%, 0.02%, and 0.05% concentrations of **AC LumiVitis** were diluted in CM and added to cells. Designated wells on each plate were incubated with CM as Untreated controls with and without light irradiation. Following an 18-hour incubation at 37°C, the media in all wells was removed and cells were washed once with PBS before fresh media was added. Next, the fibroblasts were irradiated with UV-B Light or Blue Light at the dosages outlined in Table 1. Following irradiation, the plates were incubated at 37°C for 24 hours, after which media was removed and cells were washed once with PBS. Next, 50 µL of the JC-10 Dye Solution was added to all wells and the entire plate was incubated at 37°C for 30 minutes. Lastly, 50 µL of Assay Buffer B was added to all wells and fluorescence measurements were taken at Excitation/Emission wavelengths of 490/525 nm and 540/590 nm.

Table 1. Light Wavelengths and Dosages

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

Three separate experiments were performed for each light source with conditions in duplicate and average values were recorded. Data was analyzed using a one-way ANOVA with statistical significance accepted at $p \leq 0.05$. A ratio analysis of the fluorescent intensities (525/590) was performed. The ΔΨm data is expressed as a percent of untreated fibroblasts (Complete Media) and calculated by the following equation:

$$\Delta\Psi m\ (\%\ of\ Untreated) = \frac{525/590\ Ratio_{Sample}}{525/590\ Ratio_{Untreated}} \times 100$$

Results

The data obtained met criteria for a valid assay and the controls performed as anticipated. Compared to untreated fibroblasts (Complete Media), UV-B Light and Blue Light irradiation reduced mitochondrial membrane potential. Fibroblasts treated with **AC LumiVitis** demonstrated increased ΔΨm compared to untreated fibroblasts.

ACTIVE CONCEPTS LLC
107 Technology Drive, Lincolnton | NC 28092 USA
• info@activeconceptsllc.com • Phone: +1-704-276-7100 • Fax: +1-704-276-7101 • https://activeconceptsllc.com/

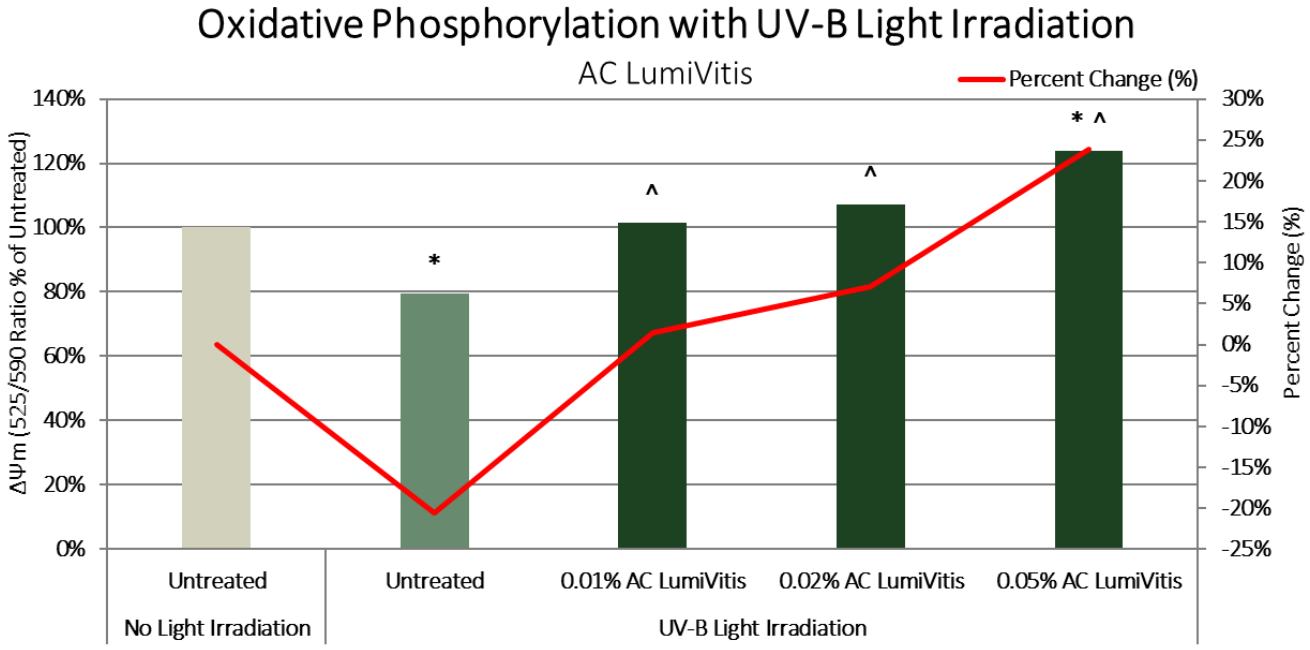

Figure 1. The Effect of UV-B Light Irradiation on ΔΨm in Dermal Fibroblasts. * indicates significance ($p \leq 0.05$) compared to untreated fibroblasts. ^ indicates significance ($p \leq 0.05$) compared to untreated fibroblasts with irradiation.

Table 2. Results from one-way ANOVA Statistical Analysis of ΔΨm 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	0.01% AC LumiVitis	0.02% AC LumiVitis	0.05% AC LumiVitis
Untreated	< 0.001*	> 0.05	> 0.05	0.001*
Untreated + Irradiated	-----	0.030^	0.014^	0.008^

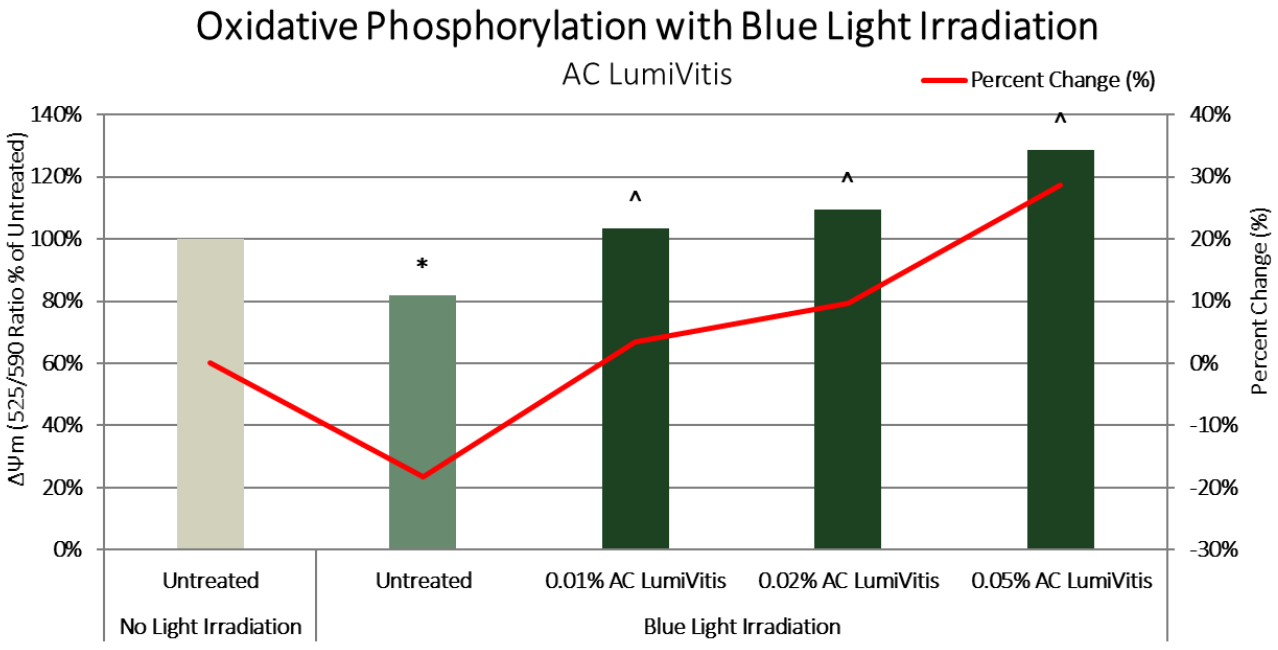

Figure 2. The Effect of Blue Light Irradiation on ΔΨm in Dermal Fibroblasts. * indicates significance ($p \leq 0.05$) compared to untreated fibroblasts. ^ indicates significance ($p \leq 0.05$) compared to untreated fibroblasts with irradiation.

ACTIVE CONCEPTS LLC
107 Technology Drive, Lincolnton | NC 28092 USA
• info@activeconceptsllc.com • Phone: +1-704-276-7100 • Fax: +1-704-276-7101 • https://activeconceptsllc.com/

Table 3. Results from one-way ANOVA Statistical Analysis of ΔΨm 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	0.01% **AC LumiVitis**	0.02% **AC LumiVitis**	0.05% **AC LumiVitis**
Untreated	0.001*	> 0.05	> 0.05	> 0.05
Untreated + Irradiated	-----	0.008^	0.009^	0.029^

Discussion

As shown in Figure 1, UV-B Light exhibited a 21% reduction in ΔΨm compared to untreated fibroblasts. Conversely, fibroblasts treated with **AC LumiViits** at 0.01%, 0.02%, and 0.05% demonstrated 1%, 7%, and 24% increases in ΔΨm compared to untreated fibroblasts, respectively (Figure 1; Table 2). Moreover, **AC LumiVitis** fully protected against UV-B Light induced reductions in ΔΨm compared to untreated fibroblasts with irradiation (Table 2). These data demonstrate **AC LumiVitis** protects ΔΨm against UV-B Light irradiation and increases oxidative phosphorylation in fibroblasts.

Similarly, fibroblasts irradiated with Blue Light exhibited an 18% reduction in ΔΨm compared to untreated fibroblasts (Figure 2). Conversely, fibroblasts treated with **AC LumiViits** at 0.01%, 0.02%, and 0.05% demonstrated 4%, 10%, and 29% increases in ΔΨm compared to untreated fibroblasts, respectively (Figure 2; Table 3). Moreover, **AC LumiVitis** fully protected against Blue Light induced reductions in ΔΨm compared to untreated fibroblasts with irradiation (Table 3). These data demonstrate **AC LumiVitis** protects ΔΨm against Blue Light irradiation and increases oxidative phosphorylation in fibroblasts.

In summary, oxidative phosphorylation is a biological process that generates ATP to assist in cellular migration and proliferation, molecular transportation across membranes, and the biosynthesis of cellular components. A necessary component of oxidative phosphorylation is the maintenance of ΔΨm given prolonged reductions in this electrochemical gradient are indicative of poor mitochondrial health. Increasing oxidative phosphorylation not only maintains ΔΨm, but also reduces the formation of reactive oxygen species which augments the ability of dermal cells to reduce characteristics of cellular aging (e.g. DNA mutation, cellular senescence, advanced glycation end products, protein oxidation, and collagen degradation). Collectively, these data indicate **AC LumiVitis** stimulates oxidative phosphorylation, demonstrated by increases in mitochondrial membrane potential, which assists in the maintenance of cellular homeostasis, vitality, mitochondrial function, and attenuate the physical signs of cellular aging.