## AC Moisture-Plex Advanced PF Efficacy Data

**Code:** 16503PF  
**INCI Name:** Glycerin & Water & Sodium PCA & Urea & Trehalose & Polyquaternium-51 & Sodium Hyaluronate  
**EINECS #:** 200-289-5 & 231-791-2 & 249-277-1 & 200-315-5 & 202-739-6 & 283-918-6 & N/A & N/A

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<td>The results indicated that effects of AC Moisture-Plex Advanced PF are dose dependent with the greatest increase in hydration achieved using the 3.0% concentration. The average increase in skin hydration using the control lotion was 1.5% after 24 hours. After 24 hours, the base lotion containing 1.0% AC Moisture-Plex Advanced PF increased skin moisturization by 5.0%, the base lotion containing 2.0% AC Moisture-Plex Advanced PF increased moisture on the skin by 8.0%, and the base lotion containing 3.0% AC Moisture-Plex Advanced PF increased moisture on the skin by 13.0%.</td>
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<td>Change in Conductance After 24 Hours</td>
<td>According to the results, the most effective level of AC Moisture-Plex Advanced PF after 24 hours is the 5.0% use level. AC Moisture-Plex Advanced PF still shows an increase at all 3 use levels after 24 hours from initial application. From these results, we can determine that there is an increase in moisture, as an increase in conductance represents a direct correlation.</td>
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<td>Comparison to Hyaluronic Acid</td>
<td>Measurements taken at 30 minutes and 2, 4, 6, and 8 hour increments showed that over these periods of time, 5.0% AC Moisture-Plex Advanced PF increased skin moisturization by an average of 61.0%, 41.0%, 21.0% 25.0% and 30.0% respectively. The 0.1% Hyaluronic Acid test sites yielded increased moisture levels by 13.0%, 11.0%, 0.0%, 5.0% and 10.0% when measured at the same time increments. The skin patches where 0.2% Hyaluronic Acid was applied also resulted moisture levels lower than those found at the AC Moisture-Plex Advanced PF sites, increase...</td>
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Version#2/06.12.18
<table>
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<td><strong>ORAC Assay</strong></td>
<td>AC Moisture-Plex Advanced PF exhibited antioxidant activity comparable to 200μM Trolox®. The antioxidant capacity of AC Moisture-Plex Advanced PF increased as the concentration increased, as a result we can assure that its ability to minimize oxidative stress is dose dependent.</td>
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<td><strong>ELISA IL-6 Assay</strong></td>
<td>AC Moisture-Plex Advanced PF exhibited anti-inflammatory effects on LPS-treated fibroblasts. This decrease in IL-6 production indicates a reduced inflammatory environment which could decrease the signs of aging and reduce the formation of fine lines and wrinkles.</td>
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<td><strong>Scratch Assay</strong></td>
<td>AC Moisture-Plex Advanced PF was able to increase cell migration and close the scratch at a rate comparable to the positive control. The mechanisms of the cells in the in-vitro scratch assay mimic the mechanisms seen in in-vivo wound healing. It can therefore be concluded that at normal use concentrations AC Moisture-Plex Advanced PF enhances healing and cell proliferation properties.</td>
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</table>
Abstract:

An impedance meter was used to determine changes in the stratum corneum following the topical application of several different lotions containing varying concentrations of AC Moisture-Plex Advanced PF.

Materials and Methods:

In a blind study 1%, 2% and 3% AC Moisture-Plex Advanced PF was added to Cetaphil Moisturizing Lotion respectively. As a control, the fourth lotion did not contain any AC Moisture-Plex Advanced PF. 10 (m/f) subjects between the ages of 24 – 61 topically applied the 4 different lotions on different skin patches twice over the 24 hour period. The lotions were labeled lotion 1 (contained 1% AC Moisture-Plex Advanced PF), lotion 2 (contained 2% AC Moisture-Plex Advanced PF), lotion 3 (contained 3% AC Moisture-Plex Advanced PF) and lotion 4 (placebo).

Prior to applying the lotion baseline stratum corneum values were determined using the DPM 9003 Impedance Meter and XPRT Software purchased from Nova Technology Corporation. Repeat measurements were taken 24 hours after the initial measurements. The efficacy of the product was determined by comparing the data obtained for each lotion to data obtained for the placebo.

Results:

<table>
<thead>
<tr>
<th>Mean % Increase Compared to Placebo</th>
<th>Lotion 1</th>
<th>Lotion 2</th>
<th>Lotion 3</th>
</tr>
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<tr>
<td>24 Hour Measurements</td>
<td>4.9</td>
<td>8.0</td>
<td>13.2</td>
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Table 1. Average moisturization percent improvement in hydration after 24 hours.
AC Moisture-Plex Advanced PF
24 Hour Moisturization Test

Discussion:

The results indicate that AC Moisture-Plex Advanced PF is capable of increasing skin moisturization after 24 hours. As expected the results indicated that effects of AC Moisture-Plex Advanced PF are dose dependent with the greatest increase in hydration achieved using the 3.0% concentration. The average increase in skin hydration using the control lotion was 1.5% after 24 hours. After 24 hours, the base lotion containing 1.0% AC Moisture-Plex Advanced PF increased skin moisturization by 5.0%, the base lotion containing 2.0% AC Moisture-Plex Advanced PF increased moisture on the skin by 8.0%, and the base lotion containing 3.0% AC Moisture-Plex Advanced PF increased moisture on the skin by 13.0% after 24 hours. These results also confirm that AC Moisture-Plex Advanced is capable of moisturizing the skin better than the control.

Graph 1. Mean percent increase in moisture measurements using 1, 2 and 3% AC Moisture-Plex Advanced PF.
Abstract

We conducted a series of tests to determine the increase in conductance of AC Moisture-Plex Advanced PF relative to increasing moisture and barrier function at different levels to determine its effectiveness after 24 hours.

Materials and Methods

The environment was kept at a constant temperature of 21°C and 19% Relative Humidity (RH). We measured the conductance on the skin of 6 volunteers before and after 24 hours by using a non-invasive method of electrical conductance across the skin via electrodes. These 6 volunteers were given 20ul of a solution of 1.0%, 5.0%, and 10.0% AC Moisture-Plex Advanced PF on the forearm.

Results

![Change in Conductance After 24 Hours](image)

Graph 1. Chance in conductance due to increase in moisture from AC Moisture-Plex Advanced PF and the effects after 24 hours.

Discussion

According to the results, the most effective level of AC Moisture-Plex Advanced PF after 24 hours is the 5.0% use level. AC Moisture-Plex Advanced PF still shows an increase at all 3 use levels after 24 hours from initial application. From these results, we can determine that here is an increase in moisture, as an increase in conductance represents a direct correlation.
Abstract

We conducted a series of tests to determine the effectiveness of AC Moisture-Plex Advanced PF at increasing moisture and barrier function in comparison to Hyaluronic Acid, a known moisturizing agent.

Materials and Methods

We measured the moisture levels of 9 volunteers before and after by using a non-invasive method of electrical conductance across the skin via electrodes. These 9 volunteers were given a solution of 5.0% AC Moisture-Plex Advanced PF, 0.2% Hyaluronic Acid, 0.1% Hyaluronic Acid, and a control vehicle on the forearm. Measurements were taken at 30 minutes, 2 hours, 4 hours, 6 hours, and 8 hours.

Results

![Moisturization Benefits of AC Moisture-Plex Advanced PF vs. Hyaluronic Acid](image)

Graph 1. Comparative increase in skin moisturization over time when using AC Moisture-Plex Advanced PF vs. Hyaluronic Acid
Discussion

According to the results, when an application of 5.0% AC Moisture-Plex Advanced PF was put on the skin, the average moisturization levels of the 9 volunteers increased significantly, +60.0%, after 30 minutes, in comparison to the 0.2% and 0.1% Hyaluronic Acid applications which increased skin moisturization by 13.0% and 11.0% respectively after the same amount of time elapsed. The measurements taken at the 2, 4, 6, and 8 hour increments showed that over these periods of time, AC Moisture-Plex Advanced PF increased skin moisturization by an average of 41.0%, 21.0% 25.0% and 30.0% respectively, whereas the 0.1% Hyaluronic Acid application only increased moisture levels by 11.0%, 0.0%, 5.0% and 10.0% when measured at the same time increments. The skin patches where 0.2% Hyaluronic Acid was applied also yielded results much less impressive than those of AC Moisture-Plex Advanced PF, measuring 10.0%, 3.0% 5.0% and 14.0% increases in moisturization at the 2, 4, 6 and 8 hour time marks, respectively. AC Moisture-Plex Advanced PF shows increased levels of moisture for a relatively prolonged period of time, even up to 8 hours after initial application.
Oxygen Radical Absorbance Capacity (ORAC) Assay

Tradename: AC Moisture-Plex Advanced PF

Code: 16503PF


Test Request Form #: 197

Lot #: 24417

Sponsor: Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092
Study Director: Erica Segura
Principle Investigator: Meghan Darley

Test Performed:
Oxygen Radical Absorbance Capacity (ORAC)

Introduction

Reactive oxygen species (ROS) are generated by normal cellular processes, environmental stresses, and UV irradiation. ROS are detrimental to cellular structures and functional molecules (i.e DNA, proteins, lipids) as they act as strong oxidizing agents or free radicals. The oxygen radical absorbance capacity (ORAC) assay is a standard method used to assess antioxidant capacity of physiological fluids, foods, beverages, and natural products. The assay quantitatively measures a sample’s ability to quench free radicals that have the potential to react with and damage cellular components.

Oxygen Radical Absorbance Capacity (ORAC) assay was conducted to assess the antioxidant capacity of AC Moisture-Plex Advanced PF.

Assay Principle

This assay is based upon the effect of peroxyl radicals generated from the thermal decomposition of 2, 2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) on the signal intensity from the fluorescent probe, fluorescein, in the presence of an oxygen radical absorbing substance. The degree of change is indicative of the amount of radical damage and the presence of antioxidants results in an inhibition in the free radical damage to the fluorescein. The antioxidant protection of the sample can be calculated by comparing it to a set of known standards. Trolox®, a water soluble vitamin E analog, with known antioxidant capabilities is used in this ORAC assay as the standard for measuring the antioxidant capacity of unknown substances. ORAC values, expressed in µM of Trolox® equivalents (TE), are calculated using the area under the curves (AUC) of the test product, Trolox®, and the control materials. Trolox equivalency is used as the benchmark for antioxidant capacity of mixtures since it is difficult to measure individual components.
Oxygen Radical Absorbance Capacity (ORAC) Assay

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Materials

A. Equipment:
Synergy H1 Microplate reader (BioTek Instruments, Winooski, VT); Gen5 software (BioTek Instruments, Winooski, VT); Pipettes

B. Buffers:
75mM Potassium Phosphate (pH 7.4); Deionized H$_2$O

C. Reagents:
2,2’-Azobis(2-methylpropionamide) dihydrochloride (AAPH) (153mM);
6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox®);
Fluorescein Sodium Salt (4nM)

D. Preparation:
Pre-heat (37°C) Synergy H1 Microplate reader; Prepare Trolox® standards, sample dilutions, fluorescein solution, and AAPH.

E. Microtitre Plates:
Corning 96 Well Black Side/Clear Bottom Microplates

Methods

Solutions of AC Moisture-Plex Advanced PF and Trolox® (positive control) were prepared in 75mM potassium phosphate buffer. Materials were prepared at three different concentrations/dilutions. Trolox® was used as a reference for antioxidant capacity and prepared at a concentrations ranging from 12.5µM to 200µM in 75mM potassium phosphate buffer.

For the ORAC assay, 25µL of test material and Trolox® were combined with 150µL of fluorescein in 75mM potassium phosphate buffer and incubated in the Synergy HT Microplate reader at 37°C for 30 minutes. At the end of the incubation period, 25µL of AAPH were pipetted into each well. Fluorescent measurements were then taken every 2 minutes for approximately 2 hours at an excitation wavelength of 485nm and an emissions wavelength of 520nm.

The AUC and Net AUC values of the standards and samples were determined using Gen5 2.0 Data Reduction Software using the below equations:

\[
AUC = 0.5 + \sum \frac{R_i}{R_1} \rightarrow \text{Where } R \text{ is fluorescence reading}
\]

\[
\text{Net AUC} = AUC_{sample} - AUC_{blank}
\]

The standard curve was obtained by plotting the Net AUC of different Trolox® concentrations against their concentration. ORAC values of samples were then calculated automatically using the Gen5 software to interpolate the sample’s Net AUC values against the Trolox® standard curve. ORAC measurements for the test material were expressed in micro moles Trolox® equivalents (µMTE), where 1 ORAC unit is equal to 1 µMTE.
Results

AC Moisture-Plex Advanced PF began exhibiting antioxidant activity at a 0.1% concentration.

![Antioxidant Capacities](image)

**Figure 1:** Antioxidant capacities

Discussion

As shown in figure 1, AC Moisture-Plex Advanced PF exhibited antioxidant activity comparable to 200µM Trolox®. The antioxidant capacity of AC Moisture-Plex Advanced PF increased as the concentration increased, as a result we can assure that its ability to minimize oxidative stress is dose dependent.

AC Moisture-Plex Advanced PF was designed to aid in barrier function, moisturization, and function as a hyaluronic acid alternative. With the present study we can confirm that this unique ingredient is not only capable of providing functional benefits but it is also capable of providing potent antioxidant benefits when added to cosmetic applications.
**Tradename:** AC Moisture-Plex Advanced PF

**Code:** 16503PF


**Test Request Form #:** 259

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

**Study Director:** Erica Segura

**Principle Investigator:** Meghan Darley

**Test Performed:**
Interleukin (IL)-6 Enzyme-Linked Immunosorbent Assay (ELISA)

**Introduction**

Interleukin-6 is a proinflammatory cytokine known to play an active role in inflammation, immunology, bone metabolism, reproduction, arthritis, neoplasia, and aging. IL-6 signals through the nuclear factor-kappa B (NF-κB) pathway that results in the transcription of inflammatory mediators, including matrix metalloproteinase-1 (MMP-1). MMP’s are responsible for breaking down the extracellular matrix and collagen in the skin leading to wrinkles, fine lines, and loss of skin elasticity. Reducing the level of IL-6 and other inflammatory mediators is believed to slow down degradation of the skin matrix and, possibly, stimulate its replenishment.

Interleukin-6 ELISA was conducted to assess the changes in IL-6 levels in AC Moisture-Plex Advanced PF-treated *in vitro* cultured human dermal fibroblasts.

**Assay Principle**

This ELISA utilizes a colorimetric reaction employing antibodies with antigen specificity to human IL-6. Monoclonal antibodies specific for IL-6 epitopes are coated on a microtiter plate. In positive samples, IL-6 will bind to these antibodies and are tagged a second time with another IL-6-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 collagen in each sample.
IL-6 ELISA Analysis

Materials

A. Kit: IL-6 ELISA Kit (Biosource; KAC1261)
B. Incubation Conditions: 37°C at 5% CO₂ and 95% relative humidity (RH)
C. Equipment: Forma humidified incubator; ESCO biosafety laminar flow hood; Microplate Reader; Pipettes
D. Cell Line: Normal Human Dermal Fibroblasts (NHDF) (Lonza; CC-2511)
E. Media/Buffers: Dulbecco’s Modified Eagle Medium (DMEM); Penicillin-Streptomycin (50U-50mg/mL); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS); Amphotericin (45pg/mL)
F. Culture Plate: Falcon flat bottom 12-well tissue culture treated plates
G. Reagents: Lipopolysaccharide (LPS) (1µg/mL)
H. Other: Sterile disposable pipette tips; wash bottles

Methods

Human dermal fibroblasts were seeded into 12-well tissue culture plates and allowed to grow to confluency in complete DMEM. 1%, 0.1%, 0.01% concentrations of AC Moisture-Plex Advanced PF were added to complete DMEM containing 1µg/mL LPS and incubated with fibroblasts for 24 hours. Complete media containing 1µg/mL LPS was used as the positive controls and complete DMEM was used a negative control.

Standards were prepared in concentrations ranging from 2476pg/mL to 0pg/mL. 50µL of Solution B was added to wells for standards and assay controls and 50µL of Solution A was added to experiment wells. 100µL of standards, controls, and samples were added to appropriate wells. After a one hour incubation at room temperature and washing, 50µL Solution A and 100µL anti-IL-6 conjugate was added to all wells. Following a one hour incubation and washing, 100 µL chromagen solution was added for the colorimetric reaction. One-hundred µL stop solution was added to stop the reaction after 15 minutes. The optical density was read at 450nm on the Synergy HT Microplate Reader.

A standard curve was created by reducing the data and generating a linear curve fit. The IL-6 concentration of AC Moisture-Plex Advanced PF treated-fibroblasts was determined by extrapolation from the standard curve and expressed in pg/mL.
Results

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

**AC Moisture-Plex Advanced PF**, at concentrations of 1%, 0.1%, and 0.01% was able to decrease IL-6 production compared to our positive control.

IL-6 levels are expressed by the following formula:

\[ \text{IL-6 Concentration} = \frac{\text{Average}_{\text{IL-6 Concentrations}} \times \text{Dilution Factor}}{\text{Dilution Factor}} \]

**Figure 1**: AC Moisture-Plex Advanced PF-treated fibroblasts IL-6 concentrations
IL-6 production percent decrease is calculated by the following formula:

\[
\text{Percent Decrease} = \left( \frac{\text{Positive Control Avg. Concentration} - \text{Sample Avg. Concentration}}{\text{Positive Control Avg. Concentration}} \right) \times 100
\]

Figure 2: Percent decrease in IL-6 production compared to positive control

Discussion

As shown in figure 1, AC Moisture-Plex Advanced PF exhibited anti-inflammatory effects on LPS-treated fibroblasts. As expected, the changes in IL-6 production using AC Moisture-Plex Advanced PF appears to be dose dependent. This decrease in IL-6 production indicates a reduced inflammatory environment which could decrease the signs of aging and reduce the formation of fine lines and wrinkles. For these reasons, we can assume AC Moisture-Plex Advanced PF is suitable for cosmetic applications designed to provide soothing and anti-aging properties.
Tradename: AC Moisture-Plex Advanced PF

Code: 16503PF


Test Request Form #: 662

Lot #: 31222

Sponsor: Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092
Study Director: Erica Segura
Principle Investigator: Meghan Darley

Test Performed: Scratch Assay

Introduction

Wounded tissue begins a complex and structured series of events in order to repair the damaged region. Some of these events include upregulation of angiogenic factors causing increased vascularization, increased deposition of extracellular matrix, and increased cell proliferation. The wound healing process begins as cells polarize toward the wound, initiate protrusion, migrate, and close the wound area. These processes reflect the behavior of individual cells as well as the entire tissue complex.

The scratch assay was conducted to assess the wound healing properties of AC Moisture-Plex Advanced PF-treated in vitro cultured human dermal fibroblasts.

Assay Principle

The in vitro scratch assay is a well-known and widely used method to study cell migration and proliferation. This assay is based on the observation that when an artificial gap or scratch is made on a confluent cell monolayer, the cells will migrate towards the opening and close the scratch. The basic steps involve creating a scratch in a cell monolayer and capturing images throughout the healing or cell migration process. Through these images we can quantify the rate of cell migration.
Materials

A. Incubation Conditions: 37°C at 5% CO₂ and 95% Relative Humidity (RH)
B. Equipment: Forma Humidified Incubator, ESCO Biosafety Laminar Flow Hood, Inverted Microscope; Camera; Pipettes
C. Cell Line: Normal Human Dermal Fibroblasts (NHDF) (Lonza; CC-2511)
D. Media/Buffers: Dulbecco's Modified Eagle Medium (DMEM); Fetal Bovine Serum (FBS); Penicillin-Streptomycin (50U-50mg/mL); Phosphate Buffered Saline (PBS)
E. Reagents: Epidermal Growth Factor-1 (100ng/mL); Paraformaldehyde (3.7%); Crystal Violet Stain
F. Culture Plate: Falcon Flat Bottom 6-Well Tissue Culture Treated Plates
G. Other: Sterile Disposable Pipette Tips; Wash Bottles; 15mL Conical Tubes

Methods

Human dermal fibroblasts were seeded into 6-well tissue culture plates and allowed to grow to confluency in complete DMEM. A 1% concentration of AC Moisture-Plex Advanced PF was added to the culture media and incubated with fibroblasts for the extent of the experiment. Epidermal Growth Factor-1 was utilized as the positive control and serum-free media (SFM) was used as a negative control. Complete media contains 10% FBS.

When cell growth reached confluency scratches were made across the well in a cross or 'X' pattern. The wells were washed with sterile PBS and fresh media containing AC Moisture-Plex Advanced PF and the controls were added. Initial images were captured immediately after the scratch took place and every 24-hours afterwards, up to 72-hours. Cells were fixed with 3.7% paraformaldehyde and stained with crystal violet for enhanced microscopy.

ImageJ software was used to analyze the images and calculate the area of the scratch and the closure rate.

Results

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

AC Moisture-Plex Advanced PF at a 1% concentration was able to increase cell migration and wound healing compared to our negative control.

Percent scratch closure and migration rate are expressed by the following formula:

\[
\frac{Scratch\ Area\ t=x - Scratch\ Area\ t=0}{Scratch\ Area\ t=0} \times 100 = \%\ Scratch\ Closure
\]

\[
\frac{Change\ in\ Area\ of\ Scratch\ (nm^2)}{Migration\ Time\ t=x} = Migration\ Rate
\]

Where \( x = time\ (hours)\ post\ scratch \)
Scratch Assay Analysis

This information is presented in good faith but is not warranted as to accuracy of results. Also, freedom from patent infringement is not implied.

Figure 1: Area of scratch

Figure 2: Percent scratch closure

Figure 3: Cell migration rate
**Scratch Assay Analysis**

Figure 4: Images at t=0 hours (A, D, G) and t=72 hours (B, E, H) for AC Moisture-Plex Advanced PF, positive control (EGF-1), and negative control (SFM). At experiment completion (t=72 hours), cells were fixed in paraformaldehyde and stained with crystal violet (C, F, I).

**Discussion**

**AC Moisture-Plex Advanced PF** (code 16503PF) was able to increase cell migration and close the scratch at a rate comparable to the positive control. The mechanisms of the cells in the in vitro scratch assay mimic the mechanisms seen in in vivo wound healing therefore we can be assured that our results are translatable outside the laboratory. It can therefore be concluded that at normal use concentrations **AC Moisture-Plex Advanced PF** (code 16503PF) enhances healing and cell proliferation properties.