Hair Pollution Protection Assay Analysis

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Tradename: AC Kerazyme® Protect

Code: 16824

CAS #: 69430-36-0 & 91771-32-3 & N/A

Test Request Form #: 6832

Lot #: 68585P

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

Test Performed:
Hair Pollution Protection Assay

Introduction

The deleterious effects of pollution in skin and hair care has become a new frontier for anti-aging active ingredients. Environmental pollutants are results of automobile exhaust gas, industrial emissions, and even emissions from simple household chores such as cooking and cleaning. Hair is subject to these environmental aggressions as well as UV irradiation and, unlike the skin, hair is quite vulnerable and lacks self-protection mechanisms. Exposure to environmental pollution can result in dry, brittle hair with decreased strength and elasticity.

Our hair pollution protection assay was conducted to assess the ability of **AC Kerazyme® Protect** to protect the hair from the oxidative effects of air pollution. Hair swatches were treated and exposed to cigarette smoke, and peroxidation of hair lipids were assessed using a Malonaldehyde (MDA) Assay. Pollutant cigarette smoke is a suitable substance containing all key pollution components such as reactive oxygen species (ROS), reactive nitrogen species, and electrophilic aldehydes. Reactive oxidants as well as free radicals from cigarette smoke are closely associated with oxidative stress and secondary oxidative events, such as lipid peroxidation.

The MDA assay is useful for quantitatively measuring the end product of lipid peroxidation and determining oxidative stress. MDA is frequently used as a bio marker for oxidative stress and, in this case, lipid peroxidation (the breaking down of lipids) due to environmental stress. An increase in MDA indicates an increase in lipid peroxidation and oxidative stress.

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Materials

A. Kit:	Lipid Peroxidation (MDA) Assay Kit (Colorimetric/Fluorometric) (ab118970)
B. Equipment:	Microplate Reader; Pipettes; Dounce Homogenizer; Cole Parmer Stable Temp BLK Heater
C. Media/Buffers:	Phosphate Buffered Saline (PBS); MDA Lysis Buffer; Glacial Acetic Acid
D. Culture Plate:	Falcon Flat Bottom 96-Well Plate
E. Reagents:	Butylated hydroxytoluene (BHT) (100X); Thiobarbituric Acid (TBA) Solution; MDA Standard
F. Other:	Sterile Disposable Pipette Tips; Virgin Hair Swatches

Methods

Testing was performed on three total virgin hair swatches. Three testing groups were observed: untreated, saturation with rinse, and saturation without rinse. For the saturation with rinse testing group, the hair swatches were fully saturated in a 5.0% **AC Kerazyme® Protect** in water solution, allowed to completely air dry, rinsed with 500 grams of deionized water, and allowed to air dry again. For the saturation without rinse testing group, the hair swatches were fully saturated in a 5.0% **AC Kerazyme® Protect** in water solution and allowed to completely air dry.

Testing was performed in a custom 22”x15”x6” smoke chamber. A standard filter pump was placed in the side of the chamber as air supply and standard ceramic filter funnel was placed in the top of the chamber for continuous exposure. Each testing group of hair was hung together in designated 3”x3” section at the top of the smoke chamber. For stagnant exposure testing, four standard 2 ounce glass retain jars were placed inside the chamber.

One hundred cigarettes were placed into the ceramic filter funnel, with the tip of the cigarettes facing outward. Cigarettes were Riverside brand and all of the cigarette filters were torn off at the base. The cigarettes stood upright tightly packed in the filter funnel and the tips were torched.

The hair samples were in the smoke chamber for a total of thirty minutes. For the first ten minutes, continuous air was pulled through the filter pump system and the cigarettes were re-torched when needed. The final twenty minutes, the hair samples underwent stagnant exposure. During stagnant exposure, five cigarettes were placed standing in each of the four retain jars and positioned at each corner of the smoke chamber and the tips of the cigarettes were lit every five minutes. During stagnant exposure, the smoke chamber was fully sealed with the exception of small opening in the top where the original filter funnel was to allow oxygen into the system with minimal smoke loss. After the hair samples completed the time in the smoke chamber, each individual hair sample was placed in a sealed plastic bag for testing.

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Hair lipid peroxidation was assessed with the Abcam Lipid Peroxidation (MDA) Assay Kit (ab118970). Samples were taken from the middle portion of the hair strand, totaling 0.2g of tissue weight. To prepare the hair for testing, each hair sample was washed with cold phosphate buffer solution (PBS) and placed in 15ml conical tubes with the MDA lysis buffer. The pestle portion of a dounce grinder and vortex were used to agitate the hair and release the lipids.

The MDA in the sample reacts with thiobarbituric acid (TBA) while being incubated on a hotplate to generate a MDA-TBA adduct. The MDA-TBA adduct can be easily quantified colorimetrically using optical density.

Results

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

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

AC Kerazyme® Protect at a concentration of 5.0% demonstrated lower levels of MDA than the control standard for both testing groups. Hair samples saturated in 5.0% **AC Kerazyme® Protect** with Rinse showed 27.0% less MDA concentration to the untreated hair sample. Hair samples saturated in 5.0% **AC Kerazyme® Protect** without Rinse showed 76.2% less MDA to the untreated hair sample.

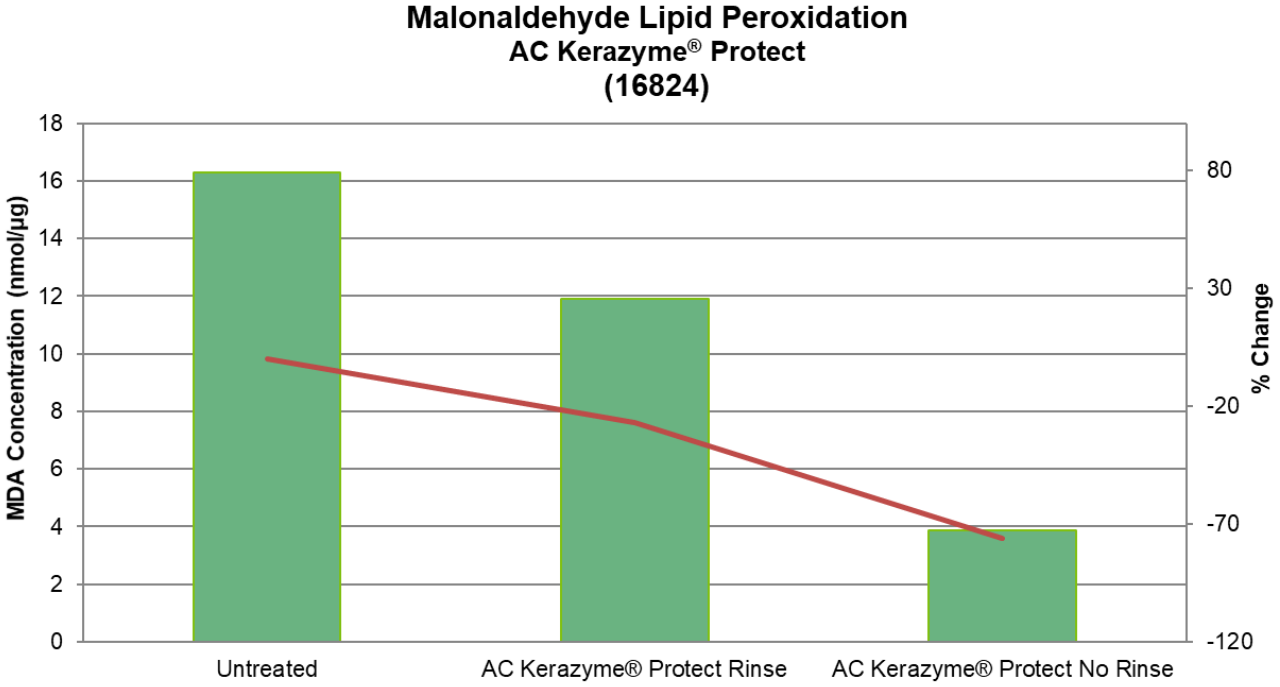

Figure 1. MDA Concentration and Percent Change

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Discussion

Pollutant cigarette smoke contains key components found in air pollution such as ROS, reactive nitrogen species, and electrophilic aldehydes. Reactive oxidants as well as free radicals from cigarette smoke are closely associated with oxidative stress and secondary oxidative events, such as lipid peroxidation. As oxidative degradation of lipids occurs, free radicals take electrons from the lipids which can lead to damage presenting as dry or brittle hair. Quantification of lipid peroxidation, which forms reactive aldehydes such as MDA, is essential to assessing oxidative stress.

In this study, **AC Kerazyme® Protect** was tested to evaluate its effects on the inhibition of lipid peroxidation of hair samples exposed to air pollution after various treatments. At a concentration of 5.0%, **AC Kerazyme® Protect** demonstrated lower levels of MDA to the untreated control for both of the treatment groups, most notably when saturated without rinse. Hair samples treated with 5.0% **AC Kerazyme® Protect** saturated without rinse showed 76.2% less MDA to the untreated hair samples.

It can therefore be concluded that at normal use concentrations **AC Kerazyme® Protect** can be used as an effective hair pollution protection ingredient, particularly when used in leave-in products.

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