AC Kerazyme®
Efficacy Data

Code: 16594
INCI Name: Hydrolyzed Keratin & Trametes versicolor Extract
CAS #: 69430-36-0 & 999999-99-4
EINECS #: 274-001-1 & 310-127-6

Name of Study	Results
Curl Retention Assay	Based on the results, it is clear from viewing the swatches that **AC Kerazyme®** is capable of retaining curls better than the unloaded vehicle seen in swatch 2. 5% **AC Kerazyme®** retained curls by 96% whereas the unloaded vehicle only retained them 72% after four hours. For this reason, **AC Kerazyme®** is an ideal ingredient to add to hair care applications designed to provide curl retention over a longer period.
Straightening Retention Assay	A half head assay indicated that **AC Kerazyme®** promotes east of styling, as 1-2 passes with the flat iron are necessary to effectively straighten curly hair. The hair was clearly smoother, straighter and healthier looking than the side that had no treatment prior to using the flat iron. **AC Kerazyme®** is ideal for the overall straightening process.
Cross Linking Assay	Shows the cross linking ability of **AC Kerazyme®** with proteins
ORAC Assay	Oxygen Radical Absorbance Capacity (ORAC) is a measure of a materials potential to protect against oxidative stress or reactive oxygen species (ROS). **AC Kerazyme®** demonstrated significant antioxidant activity by reducing the presence of ROS compared with Trolox, the vitamin E analog.

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AC Kerazyme®
Curl Retention Study

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Abstract

Unlike formaldehyde based hair systems, **AC Kerazyme®** is an all-natural ingredient that is capable of modifying hair shape while protecting it from styling damage. This unique ingredient consists of a blend of hydrolyzed keratin and *Trametes versicolor* extract. *Trametes versicolor* is a type of mushroom that contains an oxidative enzyme called laccase. This distinct heat activated enzyme cross-links free carboxylic acid groups in the hydrolyzed keratin with the amine groups along the cuticle to create a stable network that holds the hair's texture in a given shape. The purpose of this study was to determine the curl retention properties of **AC Kerazyme®.**

Materials and Methods

The humidity chamber was equilibrated two hours prior to testing at 25° C and 90% Relative Humidity. Then two swatches were washed and allowed to dry under ambient conditions. The swatches were then combed 30 times each to remove tangles. One of the swatches was treated with the unloaded vehicle (O/W emulsion) and the second swatch was treated with the vehicle containing 2% **AC Kerazyme®**. Both hair swatches were then curled with a curling iron. After completing the respective hair treatment, the tresses were hung on the support stand in the humidity chamber. Pictures were taken immediately after hanging the swatches and 4 hours later. The readings from the ruler are then converted to percent curl retention by using the following calculation:

$$\text{Percent (\%) Retention} = 100 - \left[\frac{(\text{Tress length after 4 hours} - \text{Tress length immediately after curling}) * 100}{\text{Tress length after 4 hours}}\right]$$

Results

Figure 1. Hair swatches immediately after curling

Figure 2. Hair swatches 4 hours after curling

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Percent (%) Curl Retention	
2.0% AC Kerazyme®	96.0%
Unloaded Vehicle	72.0%

Figure 3. Percent Curl Retention of **AC Kerazyme®** and the unloaded vehicle.

Discussion

Based on the results, it is clear from viewing the swatches that **AC Kerazyme®** is capable of retaining curls better than the unloaded vehicle seen in swatch 2. In fact, 2% **AC Kerazyme®** retained curls by 96% whereas the unloaded vehicle only retained them by 72% after four hours. For this reason, we can conclude that **AC Kerazyme®** is an ideal ingredient to add to hair care applications designed to provide curl retention over a longer period of time.

AC Kerazyme® Straightening Assay

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Abstract

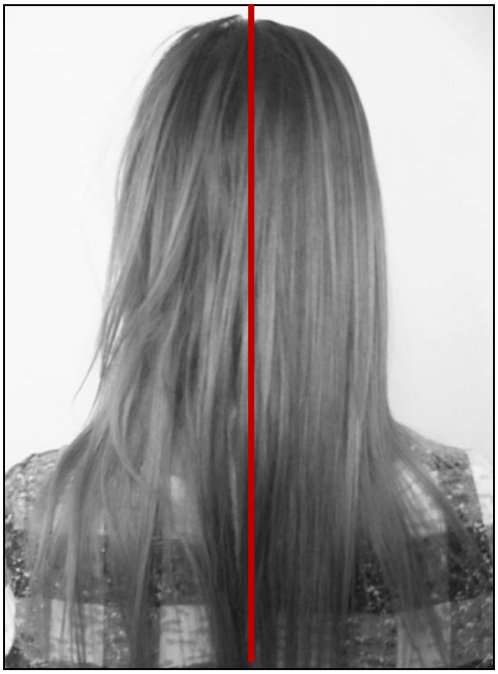
Unlike formaldehyde based hair systems, **AC Kerazyme®** is an all-natural ingredient that is capable of modifying the shape of the hair while protecting it from styling damage. This unique ingredient consists of a blend of hydrolyzed keratin and *Trametes versicolor* extract. *Trametes versicolor* is a type of mushroom that contains an oxidative enzyme called laccase. This distinct heat activated enzyme cross-links free carboxylic acid groups in the hydrolyzed keratin with the amine groups along the cuticle to create a stable network that holds the hair's texture in a given shape. Styling tools such as hot irons or blow driers can be used to activate the botanical enzyme while simultaneously straightening the hair. This all natural technology provides versatility when it comes to styling and shape retention. Our research confirms that the straightening benefits associated with **AC Kerazyme®** last until the hair is washed.

Materials and Methods

Half-head studies were conducted to determine the ability of **AC Kerazyme®** to easily promote straight styles. Panelists with naturally curly hair washed it and then were instructed to apply a 2% solution of **AC Kerazyme®** in water to one half of their head and let it air dry, while the other half of the head remained untreated. After the hair was dry, both sides were then straightened using a flat iron at 380 - 450°F (193 - 232°C).

Results

Before **After**



Natural Hair Texture - No Treatment **Flat Iron Only** **Flat Iron with 2% AC Kerazyme®**

Version#5/06-03-16

AC Kerazyme® Cross Linking Assay

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To evaluate the cross linking ability of AC Kerazyme® (16594) with proteins, we used the following procedure:

-Make a 30% Gelatin Solution in water using Low Bloom Gelatin.

-Heat solution to 80C and allow cooling to 29-32C. Check viscosity using a LV4 spindle at a 50 rpm speed. Viscosity should be in the range of 250-321 cPs. Reheat to 80C.

-Heat AC Kerazyme® (16594) to 80C.

-Allow cooling to 29-32C and check viscosity using the same LV spindle and speed. Viscosity should be in the same range as gelatin solution. Then reheat to 80C.

-Add the Gelatin Solution to the AC Kerazyme® (16594) and mix until homogenous.

-Let mixture cool to 29-32C and measure viscosity.

-Viscosity measurements should be 3 to 4 times higher than the initial measurements (800-1500 cPs range).

Composition of mixture:
86% AC Kerazyme
13% Gelatin mixture

AC Kerazyme® Straightening Assay

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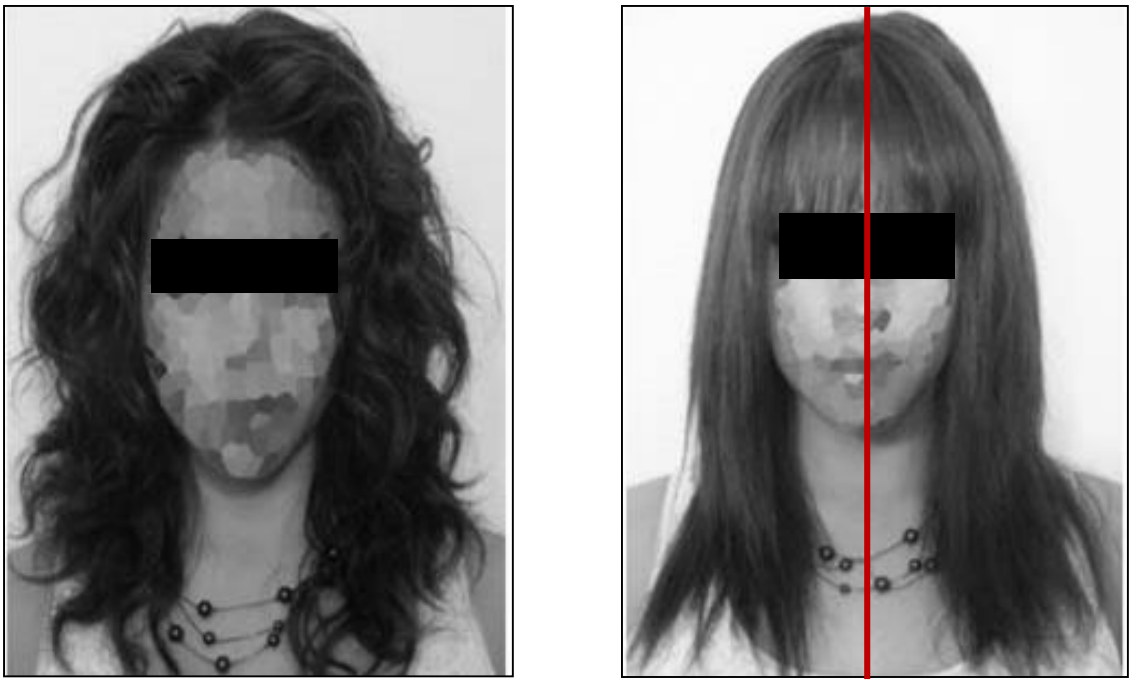
Before **After**



Natural Hair Texture No Treatment

Flat Iron with 2% AC Kerazyme®

Flat Iron Only

(Participant's Face Blurred and Eyes Covered to Ensure Privacy)

Discussion

Half head studies indicated that **AC Kerazyme®** promotes ease of styling as only 1 to 2 passes with the flat iron are necessary to effectively straighten curly hair. In the above figures, the sides treated with 2% **AC Kerazyme®** are clearly smoother, straighter and healthier looking than the sides which did not receive any type of treatment prior to using the flat iron. This demonstrates that **AC Kerazyme®** is ideal for easing the overall straightening process to achieve healthy and glamorous-looking hair.

Version#5/06-03-16

Oxygen Radical Absorbance Capacity (ORAC) Assay

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Tradename: AC Kerazyme®

Code: 165984

CAS #: 69430-36-0 & 999999-99-4

Test Request Form #: 248

Lot #: 27630

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 Kerazyme®**.

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.

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Materials

A. Equipment:	Synergy H1 Microplate reader (BioTek Instuments, Winooski, VT); Gen5 software (BioTek Instuments, Winooski, VT); Pipettes
B. Buffers:	75mM Potassium Phosphate (pH 7.4); Deionized H_2O
C. Reagents:	2,2′-Azobis(2-methylpropionamidine) 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 Kerazyme**® 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:

$$AUC = 0.5 + \frac{R2}{R1} + \frac{R3}{R1} + \frac{R4}{R1} + \cdots + \frac{Rn}{R1} \rightarrow Where\ R\ is\ fluorescence\ reading$$

$$Net\ AUC = AUC_{sample} - AUC_{blank}$$

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.

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Results

AC Kerazyme® began exhibiting antioxidant activity at a 0.1% concentration.

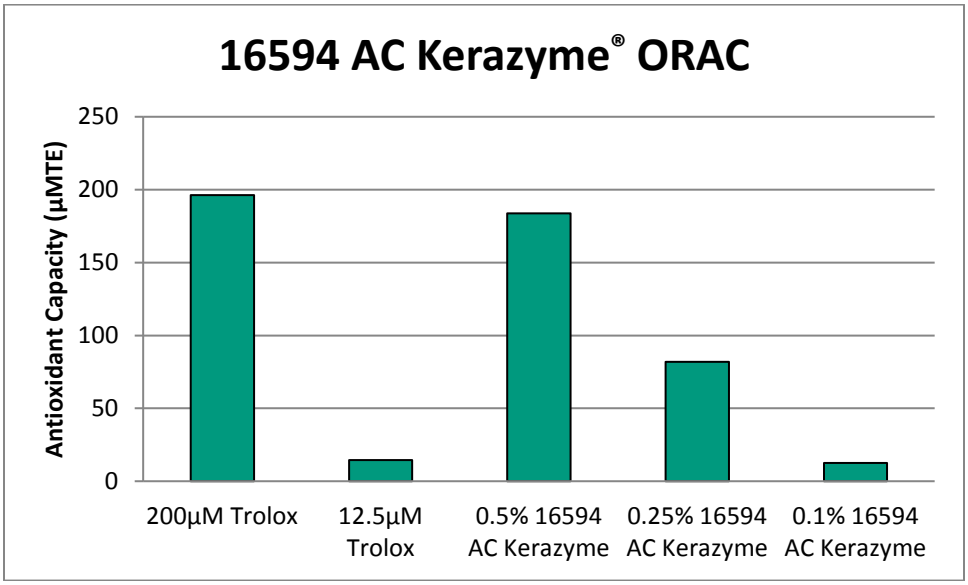

Figure 1: Antioxidant capacities

Discussion

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

AC Kerazyme® was designed for hair applications to straighten and curl and provide conditioning properties. 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.