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 Keratin Hydrolysate 30 PF

Code: 20586PF

CAS #: 69430-36-0

Test Request Form #: 14308

Lot #: S210318I

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principal Investigator: *Kara Rivera*

Test Performed:
Confocal Microscopy

Introduction

Chemical treatments such as coloring, relaxing, or perming obtain desired results by utilizing harsh chemicals that alter the internal structures in hair fibers. These treatments lead to extensive damage of hair fibers resulting in breakage, thinning, and split ends. Despite this, chemical treatments are still popular and demand for less damaging products is widespread. Therefore, cosmetic applications designed to mitigate damage and promote healthier chemically processed hair are critical.

Keratin and lipids are essential to maintaining healthy hair structure and integrity. Damage to these key components caused by chemical treatments results in brittle, dry hair. Confocal Microscopy imaging allows visualization and quantification of hair keratin and lipids via changes in fluorescence. A product that provides protection against chemical treatments will maintain fluorescent levels similar to untreated hair.

Accordingly, Confocal Microscopy was conducted to assess the ability of **AC Keratin Hydrolysate 30 PF** to protect hair against chemical treatments.

Assay Principle

Virgin human hair tresses were tested with a 40 V bleach chemical treatment to understand the protective capability of a cosmetic product. After treatment, tresses were treated with cosmetic products and examined using confocal microscopy examination to visualize the extent of damage. Images provide qualitative and quantitative damage of the hair fibers.

Confocal Microscopy employs a laser and fluorescent technology to capture a series of high-resolution 2D photos that layer to form a 3D appearance. A Nile Red fluorescent probe was used to visualize damage as its excitation and emission spectra are dependent on the surrounding chemical environment and shifts to longer wavelengths with increasing polarity. Hair lipids generate a strong polar region shifting the Nile Red emission color to red, whereas non-polar keratin will display a green color. Accordingly, Green Fluorescent Protein (GFP) and Rhodamine (Rhod) filters were employed to visualize keratin and lipids, respectively.

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Materials

A. **Hair Samples:** Human Virgin Brazilian Hair Tresses
B. **Products:** Paul Mitchell The Color Cream Developer 40 V*

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

Methods

Three virgin hair tresses were collected and assigned to each condition described in Table 1. 40 V developers were mixed with bleach to a "butter cream" consistency. Two tresses were treated with bleach according to product directions, after which the tresses were thoroughly rinsed. Next, tresses were treated with 2.0% **AC Keratin Hydrolysate 30 PF**, or left untreated, then rinsed again and allowed to air dry fully. The third tress was left unbleached and treated as the Untreated Virgin Control.

Table 1. Descriptions of the Conditions for each 40 V Brunette Hair Tresses.

Conditions
Untreated Virgin Control
40 V Bleach
40 V + 2.0% **AC Keratin Hydrolysate 30 PF**

Hair tresses were exposed to a 0.1% Nile Red solution in 1:1 methanol/chloroform for 1 hour. Excess moisture was wicked off, and hair was placed directly into molds containing 100% EPON (an epoxy resin). Samples were baked at 60°C overnight. The lab cut cross sections at 15 microns with a Leica EM UC7 ultramicrotome. Cross-sections were dried onto slides then mounted with coverslips using Permount. Images were captured at 63X using a Zeiss LSM 700 Laser Scanning Confocal Microscope with excitation/emission wavelengths of 488 nm/520 nm (green fluorescent protein - GFP) and 555 nm/619 nm (rhodamine filter - Rhod.). IMARIS software was used for immediate image analysis and processing. Mean fluorescent intensity (MFI) data were analyzed with ImageJ (NIH) to quantify the visible damage caused by bleach. Ten individual fibers were selected at random from each sample for imaging.

Results

The data obtained met criteria for a valid assay and the controls performed as anticipated. Compared to the Untreated Virgin Control, hair treated with 40 V Bleach experienced damage with reduced keratin and lipid expression. Conversely, tresses treated with **AC Keratin Hydrolysate 30 PF** after chemical treatments demonstrated less keratin and lipid damage compared to the chemically treated controls.

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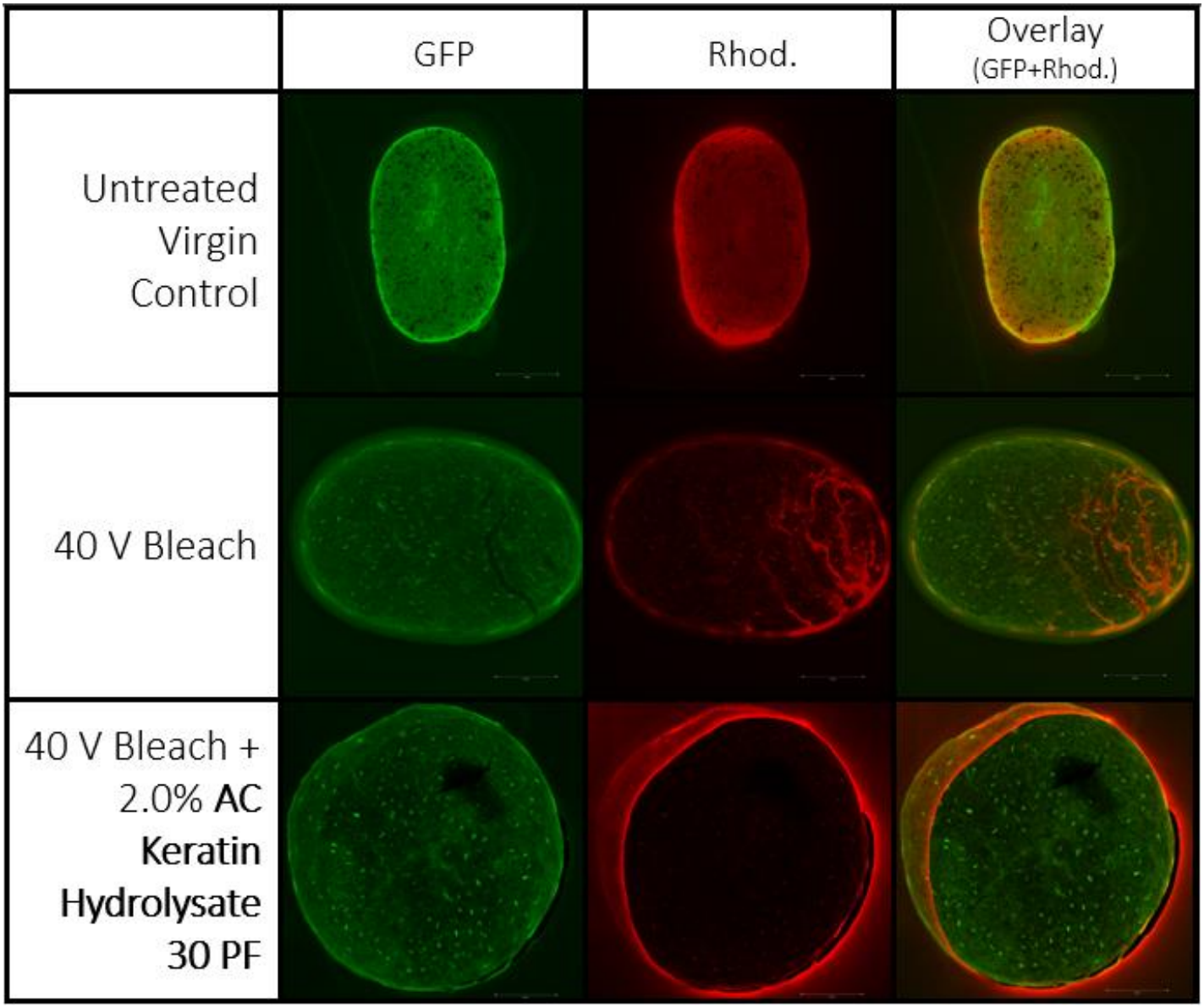

Figure 1. Representative Confocal Images of Hair Fibers Treated with 40 V Bleach. Green Fluorescent Protein (GFP) indicates keratin and Rhodamine (Rhod) indicates lipids.

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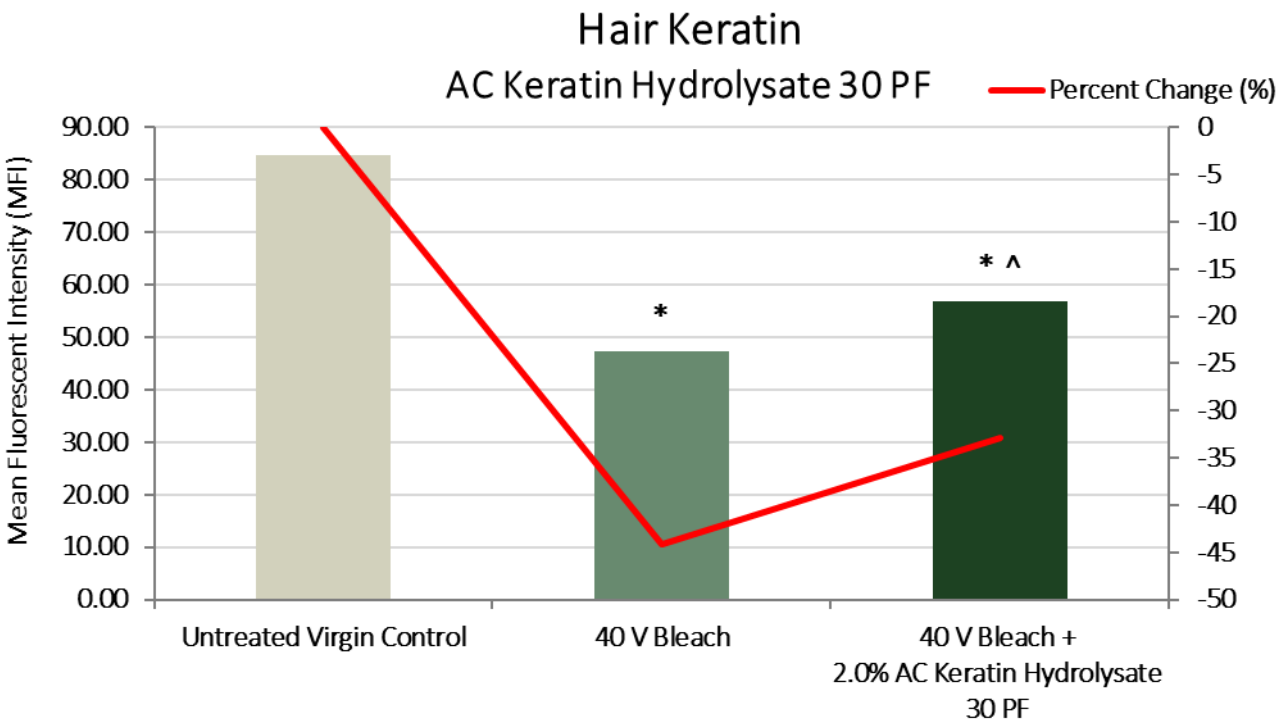

Figure 2. Keratin Intensity (GFP) of Hair Fibers Treated with 40 V Bleach. * indicates significance ($p \leq 0.05$) compared to Untreated Virgin Control. ^ indicates significance ($p \leq 0.05$) compared to 40 V Bleach.

Table 2. Results from one-way ANOVA Statistical Analysis for Hair Keratin Intensity. Results represent p-values between conditions compared. * indicates significance ($p \leq 0.05$) compared to Untreated Virgin Control. ^ indicates significance ($p \leq 0.05$) compared to 40 V Bleach.

	40 V Bleach	2.0% AC Keratin Hydrolysate 30 PF
Untreated Virgin Control	< 0.001*	0.001*
40 V Bleach	-----	0.033^

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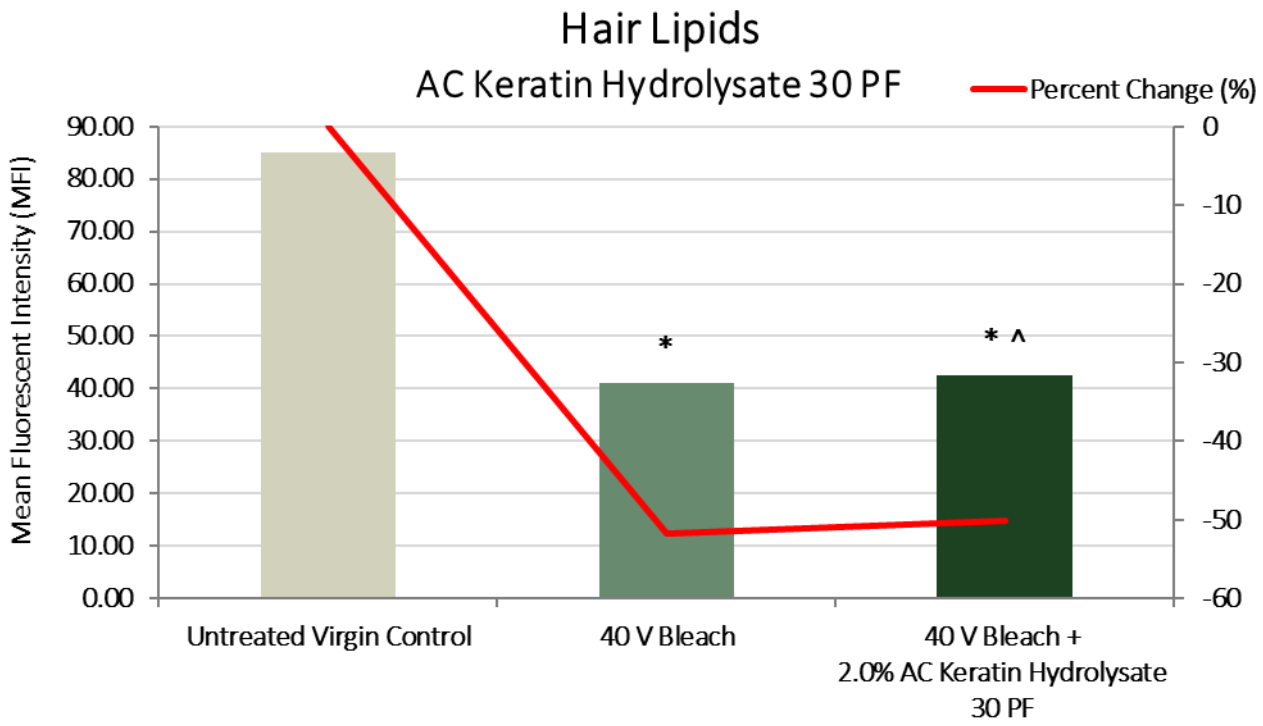

Figure 3. Lipid Intensity (Rhod.) of Hair Fibers Treated with 40 V Bleach. * indicates significance ($p \leq 0.05$) compared to Untreated Virgin Control.

Table 3. Results from one-way ANOVA Statistical Analysis for Hair Lipid Intensity. Results represent p-values compared to Untreated Virgin Control. * indicates significance ($p \leq 0.05$) compared to Untreated Virgin Control.

	40 V Bleach	2.0% AC Keratin Hydrolysate 30 PF
Untreated Virgin Control	< 0.001*	0.001*
40 V Bleach	-----	0.045^

Discussion

As demonstrated in Figures 1-3, the 40 V Bleach caused visible and quantifiable damage to the hair tresses. Compared to the Untreated Control, the 40 V Bleach tress experienced significant decreases in keratin and lipids of 44% and 52%, respectively, compared to the Untreated Virgin Control (Tables 2 and 3). This data demonstrates that bleach treatments on hair cause significant damage to the hair fiber cuticle.

Alternatively, hair fibers treated with 2.0% AC Keratin Hydrolysate 30 PF only experienced decreases in keratin and lipids of 33% and 50%, respectively, compared to the Untreated Virgin Control, and exhibited significantly higher levels of both compared to the tresses treated with 40V bleach alone (Tables 2 & 3). This data indicates AC Keratin Hydrolysate 30 PF protects hair against 40 V bleach developer induced damage.

Collectively, these results indicate AC Keratin Hydrolysate 30 PF provides protective capabilities when used after hair chemical hair treatments by maintaining keratin and lipid content. In summary, AC Keratin Hydrolysate 30 PF protects hair from harsh bleaching without influencing the desired results when used at recommended use level.