Active Concepts
Tomorrow's Vision... *Today!®*

ProCutiGen® Thermal Shield Efficacy Data

Code: 20828
INCI Name: Hydrolyzed Keratin
CAS #: 69430-36-0
EINECS #: 274-001-1

Type of Study	Results
HIROX 3D Imaging	Within the images, significantly less damage can be viewed on both the Untreated Virgin swatch and **ProCutiGen® Thermal Shield** treated swatch. Whereas the water treated swatch exhibits significantly more damage visually. In addition to the visual evidence, the photos were quantified via histograms based on luminescence. This clearly depicts the ability of **ProCutiGen® Thermal Shield** to protect the hair fiber reducing overall damage to the fiber.
Scanning Electron Microscopy	When the untreated images are compared to the **ProCutiGen® Thermal Shield** treated swatches, a significant decrease in damage of the cuticle is exhibited. Better yet, the **ProCutiGen® Thermal Shield** treated SEM images depict the creation of a de-novo cuticle on the damaged cuticle.
Thermal Protection Assay	The hair samples treated with ProCutiGen® Vegan Thermal Shield had less protein loss, indicating that there was less damage to the hair cuticle. This data supports that by forming a de novo cuticle on the hair shaft, **ProCutiGen® Thermal Shield** is able to proactively protect the hair cuticle.
Tensile Strength Data	Parameters tested within this set of data are solely based on linear stress applied to the hair. Linear stress applied as a direct parallel force is not the ideal measure of real word stress and strain applied to the hair on a daily basis. In turn, **ProCutiGen® Thermal Shield** does not have an effect on this parameter nor do the claims associated with **ProCutiGen® Thermal Shield** relate to this testing.

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Hirox 3D Imaging

Tradename: ProCutiGen® Thermal Shield

Code: 20828

CAS #: 69430-36-0

Test Request Form #: 3299

Lot #: NC170406-F

Test Performed:
Hirox 3D Imaging

Background

Everyday stressors come in all forms whether environmental, chemical, or thermal. Rather than focusing on repairing broken bonds that occur during physical and thermal stress, **ProCutiGen® Thermal Shield** consists of bivalent cationic peptides that create a *de novo* cuticle on the hair to prevent damage from happening in the first place.

This study was conducted to determine if **ProCutiGen® Thermal Shield** is capable of protecting the hair when thermal styling stress is applied.

Methods & Materials

This study was conducted by salon professionals using Sensationnel Bare & Natural Brazilian 100% Virgin Remi Unprocessed Human Hair (Hair Zone Moonachie, NJ). One swatch, left unaltered, was analyzed as the control. Two test swatches were treated and submitted for testing. One swatch was treated by spritzing with water, blown dry for two minutes, and flat ironed at 450°F, 5 run throughs. The other test swatch was treated, spritzed with a 2.0% **ProCutiGen® Thermal Shield** solution and water, blown dry for two minutes, and flat ironed at 450°F, 5 run throughs. The swatch treatment was designed to mimic everyday effects of styling the hair. It is important to note no additives or fixatives were used in the test solution. This was done intentionally in order to visually see clear results.

Manufacturing Solutions Center (MSC) located in Conover, North Carolina was asked to perform Hirox 3D Imaging on the five hair swatches provided by Active Concepts, LLC. MSC utilized a KH-7700 Hirox 3D Imaging Microscope to perform the test. The lens used was MX(G)-5040Z with magnification ranging from 50x-300x.

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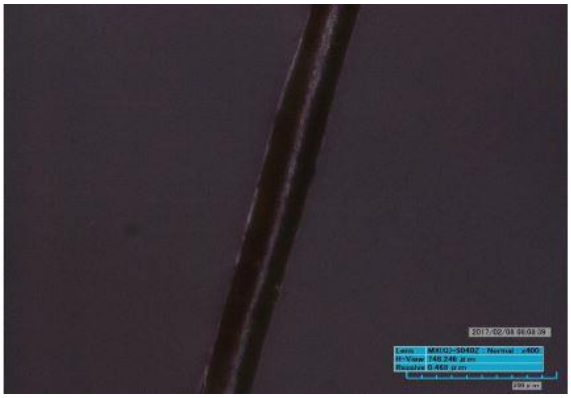
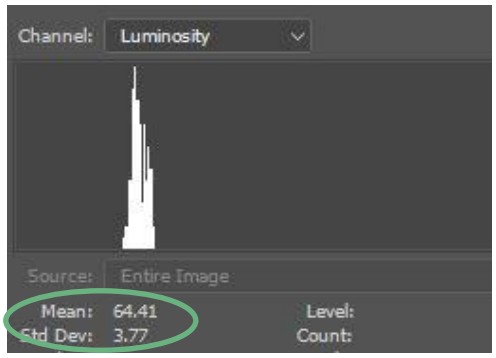
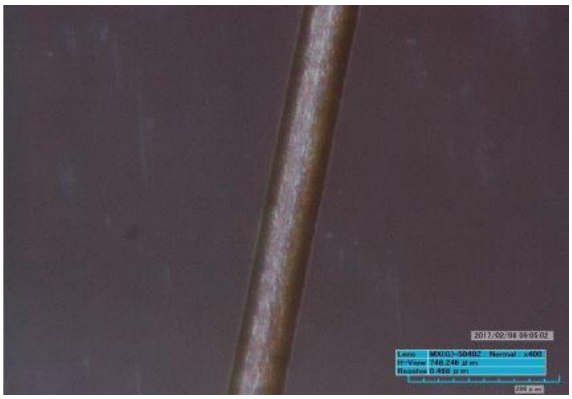
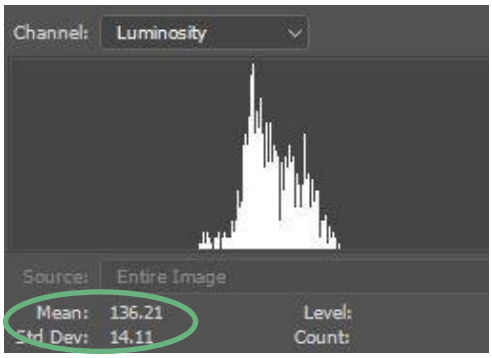
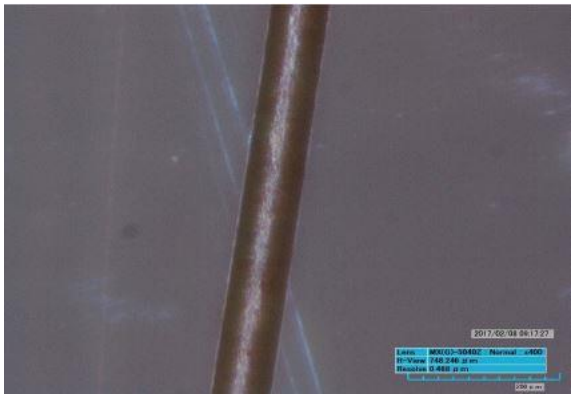
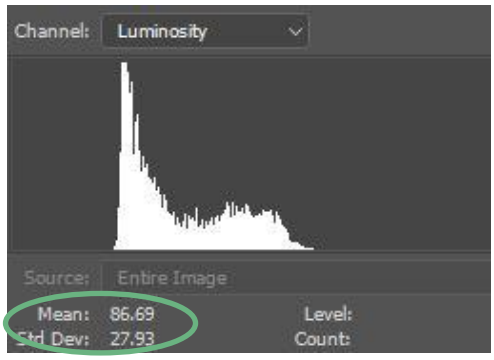
Results

Swatch Description	HIROX Image	Histogram Quantification
Untreated Virgin Hair		
Water + Blown Dry & Flat Ironed		
Water + **20830 ProCutiGen® Thermal Shield** + Blown Dry & Flat Ironed		

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Discussion

Hirox 3D Microscopic Examination is a test method for microscopic examination of hair samples. Damage of the hair fiber can be seen within these images in which the damaged areas of the fiber fluoresce. The more fluorescence a fiber exhibits, the more damaged. Within the images above significant less damage can be viewed on both the Untreated Virgin swatch and **ProCutiGen® Thermal Shield** treated swatch. Whereas the water treated swatch exhibits significantly more damage visually. In addition to the visual evidence, the photos were quantified via histograms based on luminescence. The values denoted clearly depict the ability of **ProCutiGen® Thermal Shield** to protect the hair fiber reducing overall damage to the fiber after being exposed to thermal styling stress. **ProCutiGen® Thermal Shield** consists of bivalent cationic peptides that create a *de novo* cuticle on the hair to prevent damage from happening in the first place.

Scanning Electron Microscopy

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Tradename: ProCutiGen® Thermal Shield

Code: 20828

CAS #: 69430-36-0

Test Request Form #: 3299

Lot #: NC170406-F

Test Performed:
Scanning Electron Microscopy (SEM)

Background

Everyday stressors come in all forms whether environmental, chemical, or thermal. Rather than focusing on repairing broken bonds that occur during physical and thermal stress, **ProCutiGen® Thermal Shield** consists of bivalent cationic peptides that create a *de novo* cuticle on the hair to prevent damage from happening in the first place.

This study was conducted to determine if **ProCutiGen® Thermal Shield** is capable of protecting the hair when thermal styling stress is applied.

Methods & Materials

This study was conducted by salon professionals using Sensationnel Bare & Natural Brazilian 100% Virgin Remi Unprocessed Human Hair (Hair Zone Moonachie, NJ). One swatch, left unaltered, was analyzed as the control. Two test swatches were treated and submitted for testing. One swatch was treated by spritzing with water, blown dry for two minutes, and flat ironed at 450°F, 5 run throughs. The other test swatch was treated, spritzed with a 2.0% **ProCutiGen® Thermal Shield** solution and water, blown dry for two minutes, and flat ironed at 450°F, 5 run throughs. The swatch treatment was designed to mimic everyday effects of styling the hair. It is important to note no additives or fixatives were used in the test solution. This was done intentionally in order to visually see clear results.

Gaston College Textile Technology Center located in Belmont, North Carolina was asked to perform Scanning Electron Microscopy Imaging (SEM) on the swatches provided by Active Concepts, LLC. Gaston College used a Zeiss DSM 962 to perform the test at 20.0kV using a magnification range from 50x-300x. This method utilizes an electron microscope that produces images a chemically treated hair by scanning the hair with a focused beam of electrons. These electrons interact with the atoms of the hair sample to provide images of the hairs surface topography and surface composition.

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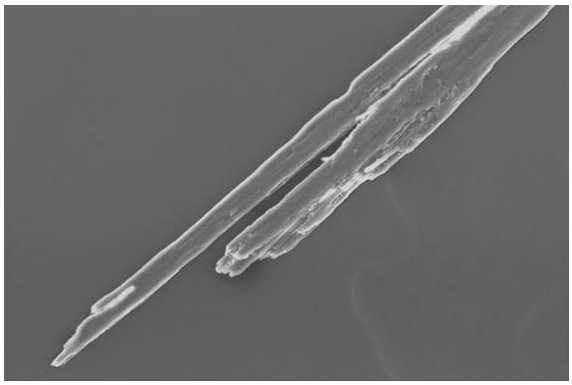
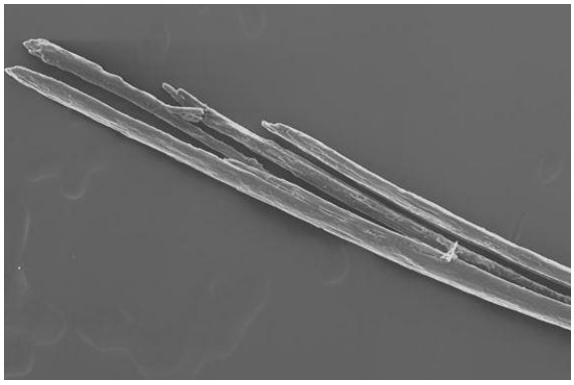
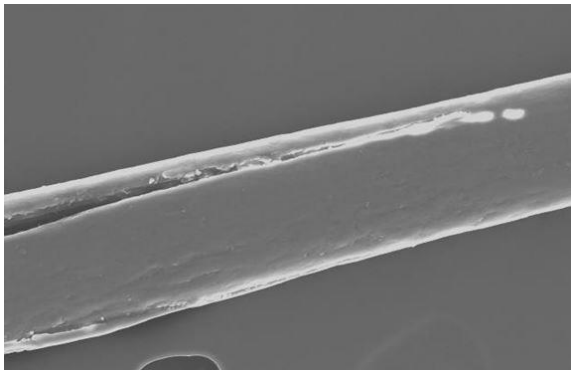
Results

Swatch Description	SEM Image
Untreated Virgin Hair	
Water + Blown Dry & Flat Ironed	
Water + **20828 ProCutiGen® Thermal Shield** + Blown Dry & Flat Ironed	

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Discussion

Standard Electron Microscopy (SEM) imaging shows high resolution images of the hair cuticles of each hair swatch. The SEM images depict how the outermost layer of the hair, the cuticle, is effected by stressors, in this case thermal styling stressors. The SEM imaging demonstrates that, Untreated Virgin hair is clearly already prone to damage from everyday aggressors, showing characteristic signs of breakdown. The imagery results of the water treated, curled sample depict an extensively damaged, split cuticle. This type of damage leads to irregular growth, breakages, and overall unhealthy, dead appearance. At a singular level, one cuticle may not seem important, but these strand to strand imperfections contribute to a much bigger picture of unhealthy and unprotected hair. When the untreated images are compared to the **ProCutiGen® Thermal Shield** treated swatches, a significant decrease in damage of the cuticle is exhibited. Better yet, the **ProCutiGen® Thermal Shield** treated SEM images depict the creation of a *de-novo* cuticle on the damged cuticle. The cuticle corrects and acts as a protective layer to the fiber. The cuticle formation **ProCutiGen® Thermal Shield** employs also exhibits properties such as moisturization, pH balance, barrier protection, and additionally, protection from hair weakening after exposure to heat rendering **ProCutiGen® Thermal Shield** the ideal addition to everyday treatment to repair and protect against everyday stressors as well as heat styling stressors.

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Thermal Protection Assay

Tradename: ProCutiGen® Thermal Shield

Code: 20828

Test Request Form #: 3154

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Test Performed:
Hair Protein Extraction
Bradford Protein Assay
Protein Gel Electrophoresis

Introduction

Hair fibers generally consist of three distinct morphological components, the outer protective layers known as the cuticle, the major structural components, or the cortex, and the porous components, or the medulla. The cuticle plays an important role both as a protective barrier and for many of the cosmetic properties of the hair, whereas the cortex provides mechanical strength to the hair fiber as a whole. It is known that the physicochemical properties of hair change as a result of damage to hair. Quantitative measurements in the amount of protein removed from hair during heat styling can serve as a method to assess hair damage. Hair protein extraction, Bradford Protein analysis, and protein gel electrophoresis were performed in this Thermal Protection Assay to determine the ability of **ProCutiGen® Thermal Shield** to protect hair from heat styling damage.

Hair Swatch Treatment Materials & Methods

This study was conducted by salon professionals using Sensationnel Bare & Natural Brazilian 100% Virgin Remi Unprocessed Human Hair (Hair Zone Moonachie, NJ). A total of six hair swatches were used and submitted for testing. The treatment for each hair swatch is detailed below. The virgin untouched and bleach untouched hair swatches served as controls.

Treatment Groups:

1. **Virgin ProCutiGen® Thermal Shield-Treated, Flat Ironed**
    a. Virgin Hair spritzed with a 2.0% **ProCutiGen® Thermal Shield** in water solution, blown dry for two minutes, and flat ironed at 450°F for 5 passes.
2. **Bleached ProCutiGen® Thermal Shield-Treated, Flat Ironed**
    a. Virgin hair bleached with 30V Pravana bleach, washed, and blown dry. Hair swatch was then spritzed with a 2.0% **ProCutiGen® Thermal Shield** in water solution, blown dry for two minutes, and flat ironed at 450°F for 5 passes.
3. **Virgin Water-Treated, Flat Ironed**
    a. Virgin Hair spritzed with water, blown dry for two minutes, and flat ironed at 450°F for 5 passes.

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4. **Bleached Water-Treated, Flat Ironed**
    a. Virgin hair bleached with 30V Pravana bleach, washed, and blown dry. Hair swatch was then spritzed with water, blown dry for two minutes, and flat ironed at 450ºF for 5 passes.
5. **Virgin Untouched**
    a. Virgin hair, no treatment.
6. **Bleached Untouched**
    a. Virgin hair bleached with 30V Pravana bleach, washed, and blown dry.

Hair Protein Extraction

Materials

A.	**Kit:**	Minute™ Protein Extraction Kit for Hair and Nails (HD-021 Invent Biotechnologies, Inc.)
B.	**Equipment:**	Microcentrifuge; Pipettes; Vortex; Bead bath
C.	**Reagents:**	Kit Buffers, 10mM Dithiothreitol (DTT)

Methods

4 mg of each treatment group were finely chopped (1-2 mm or smaller) and placed into a 2 ml microcentrifuge tube. 400µL of buffer A was added to the tube followed by 20µL of 10mM DTT acting as a reducing agent. A pipette tip was used to disrupt the tissues and ensure all tissue was filly submerged in the buffer solution. The tubes were then incubated a 55 °C for 24 hours. 40µL of buffer B was added to the tube and vortexed briefly. All contents were poured into a filter cartridge, using a 200µL pipette tip to push all material into the filter cartridge. The tube was then placed in the microcentrifuge for 3 minutes at 13,000g. The supernatant of the flow through containing the extracted proteins was then transferred to a fresh tube. The protein samples were then used for downstream application in the Bradford protein assay for protein content quantification and gel electrophoresis for molecular weight visualization and quantification.

Bradford Protein Assay

Equipment

UV/Vis Spectrophotometer: UV-1800 (Shimadzu)
Cuvettes (reagents stain the cuvettes wash immediately)

Reagents

1. Bradford reagent: Sigma Aldrich (0.1-1.4mg/ml) (B6916)
2. Buffer System: The sample solvent system is used (DI water is most common)
3. BSA solution: 2.0 mg/ml- Sigma Aldrich (PO834)

Assay

1. Warm up spectrophotometer and blank with water.

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2. Into four separate test tubes aliquot 12.5, 25, 50, 70 µL of BSA solution. Bring the volume of each to 100 µL with DI H2O.
3. Prepare the blank solution: 100 µL DI H2O, place into separate test tube.
4. To each test tube add 3 ml Bradford Reagent and vortex. Let stand at room temperature for approximately 5 minutes. (Protein-dye complex is stable up to 60 minutes)
5. Measure absorbance at 595 nm.
6. Generate a standard curve by plotting Absorbance (A595) versus protein concentration.
    a. 0, 250, 500, 1000, and 1400 µL l/mg
7. For unknowns: repeat steps 1-5 using the unknown sample in place of the BSA. Plot the A595 and use the standard curve to determine protein content of unknowns.

Protein Gel Electrophoresis

Protein gel electrophoresis provides both quantitative as well as qualitative data for protein samples of interest

Materials

D. Run Conditions:	100 Volts for 75 Minutes
E. Equipment:	Mini Gel Tank (Invitrogen); Power Supply (Hoefer); Pipettes
F. Gel:	10-well 14% Polyacrylamide
G. Reagents:	SureCast Acrylamide 40%; SureCast Resolving Buffer; Distilled Water; 10% SureCast APS; SureCast TEMED; Simply Blue SafeStain; NuPAGE SDS Running Buffer; Native Tris-Glycine Sample Buffer (2X)
H. Other:	Spectra Multicolor Broad Range Protein Ladder 260-10 kDa (Thermo Scientific); Spectra Multicolor Low Range Protein Ladder 42-1.7 kDa (Thermo Scientific)

Methods

The SureCast plate and gel casting system was used to prepare the polyacrylamide gels. The gels were prepared using the specifications listed in the SureCast system guidelines specific to the particular grade gel chosen for the sample type. A 10-well comb was used to create wells for up to 10 samples to be loaded. Once the gels had hardened, the comb was removed and the gels were rinsed twice with 1X NuPAGE SDS Running Buffer. The casting plates were locked into the mini gel tank and the remaining running buffer was used to fill the tank. The samples were diluted in a 1:8 sample: loading buffer ratio and 15 µL of each sample mixture were added to the designated wells. 5 µL and 10 µL of the low molecular weight and high molecular weight standards, respectively, were added to the designated wells.

The gel was run for 75 minutes at 100V, removed from the glass casting plates, and rinsed with distilled water three times for five minutes each rinse with slight agitation. The gel was then fully submerged in the Simply Blue SafeStain for 2.5 hours with intermittent agitation and then rinsed in distilled water for up to 1 hour and gel images were taken.

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Results

As seen in Figures 1 and 2, each of the virgin hair samples had lower extractable protein concentrations than their bleached counterpart. Bleaching and heat styling breaks down the protein in the hair fiber and allows for a greater concentration of protein to be extracted. The results in Figures 1 and 2 demonstrate an increase in extractable protein obtained through bleaching and heat treatment. The application of 2.0% **ProCutiGen® Thermal Shield** to both virgin and bleached hair followed by flat ironing helped to decrease the amount of protein lost, when compared to the virgin and bleach hair treated with water and flat ironed. As demonstrated in Figure 3, the application 2.0% **ProCutiGen® Thermal Shield** to virgin hair retained 60.9% more protein concentration during heat styling compared to water alone. For bleached hair, the application of 2.0% **ProCutiGen® Thermal Shield** before heat styling allowed the hair to retain 65.4% more protein, when compared to water alone.

Hair Sample	Protein Loss (mg/ml)
Virgin **ProCutiGen® Thermal Shield**-Treated, Flat Ironed	0.56
Bleached **ProCutiGen® Thermal Shield**-Treated, Flat Ironed	1.04
Virgin Water-Treated, Flat Ironed	1.05
Bleached Water-Treated, Flat Ironed	2.05
Virgin Untouched	0.8
Bleached Untouched	1.55

Figure 1. Concentration of extractable protein for each hair sample.

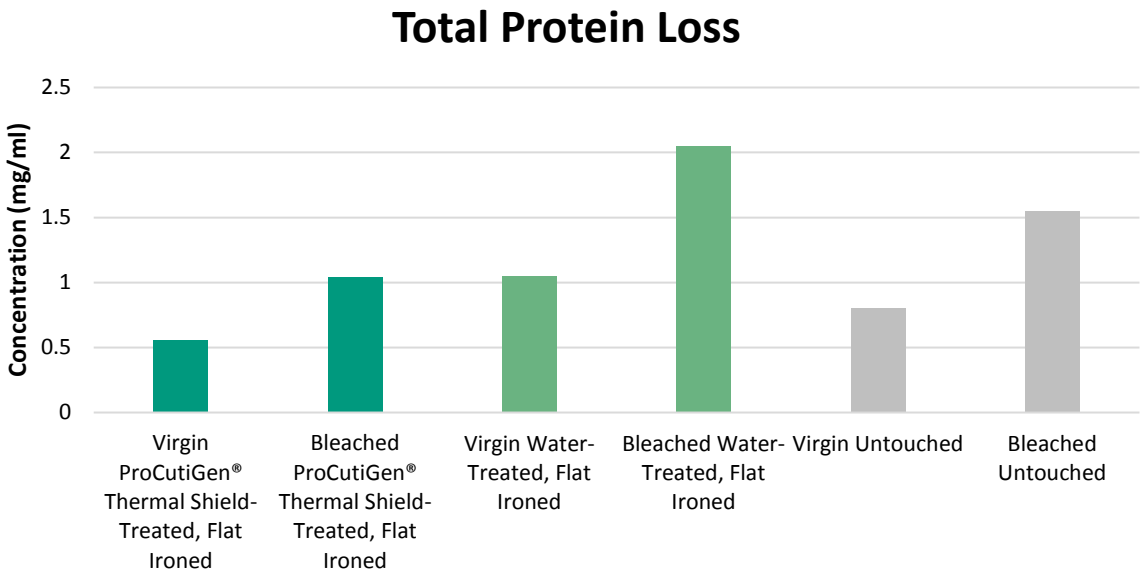

Figure 2. Concentration of extractable protein for each hair sample.

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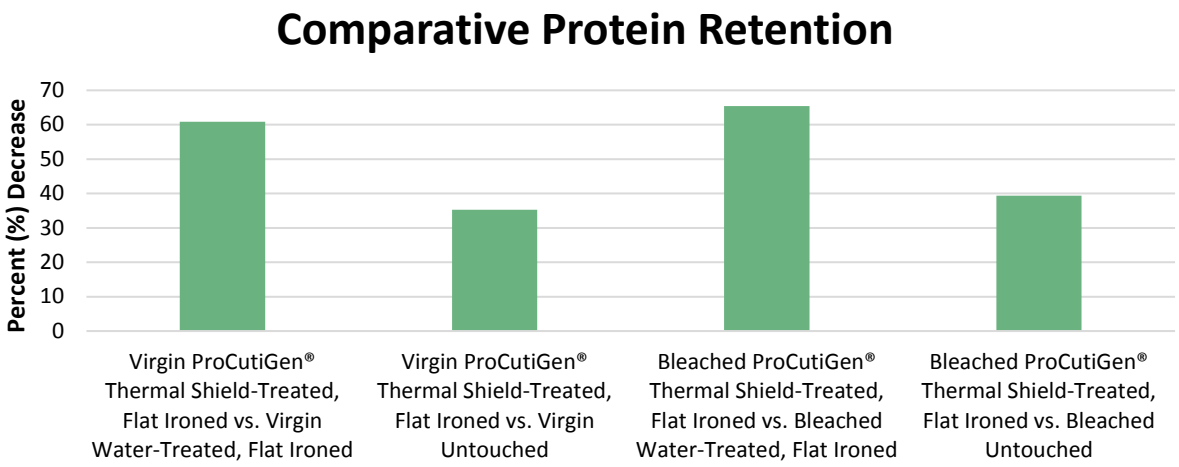

Figure 3. Comparative protein retention.

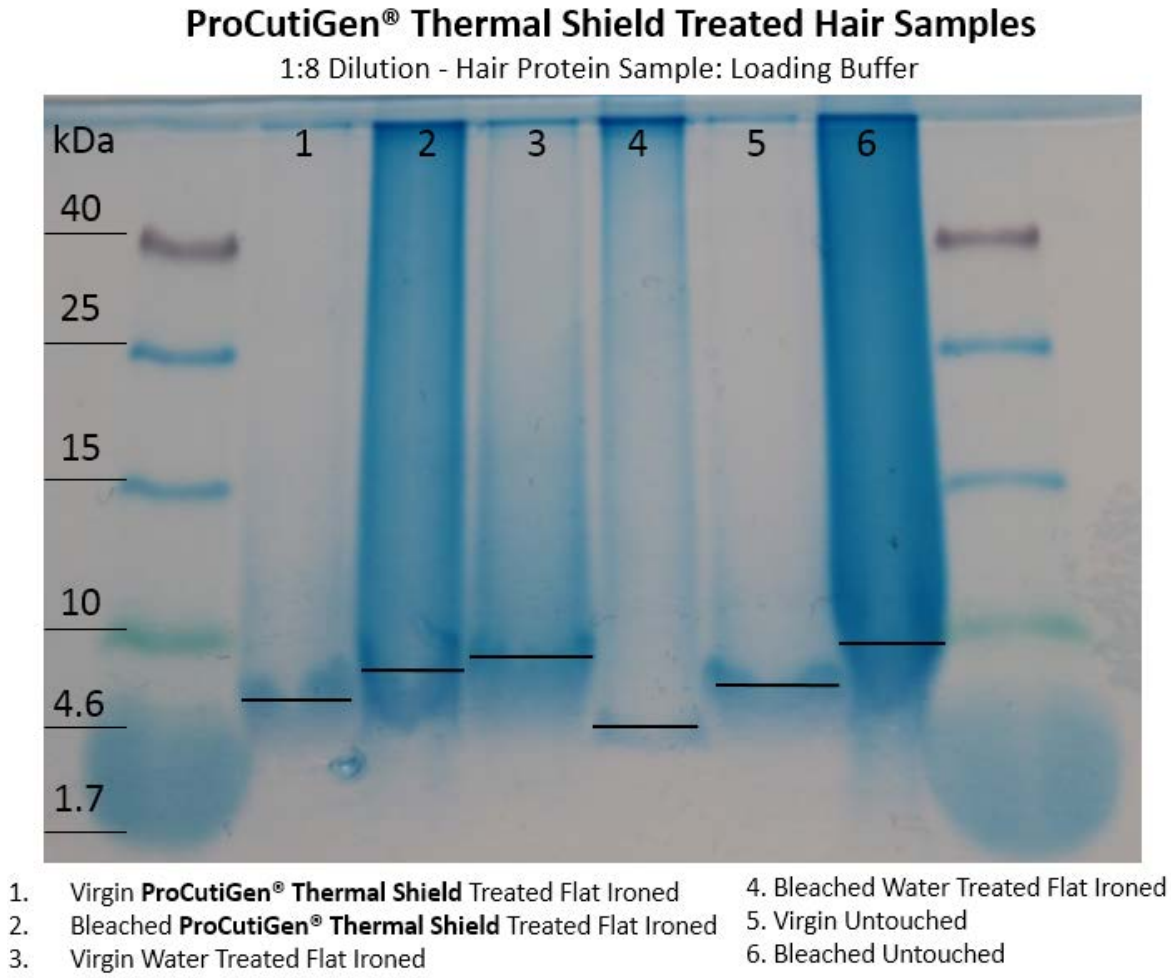

Figure 4. Protein gel electrophoresis of hair samples.

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In Figure 4, the bleached hair samples in lanes 2, 4, and 6, exhibit an increased dye density. This increased dye density correlates a higher amount of protein loss and consequential damage. In hair samples with less damage, such as the virgin hair samples in lanes 1, 3, and 5, the hair follicle is less porous and releases a lower concentration of protein.

Discussion

It is important to note that the application of 2.0% **ProCutiGen® Thermal Shield** to both virgin and bleached hair prior to heat styling produced lower extractable protein concentrations than the virgin untouched and bleached untouched samples, respectively. The application of 2.0% **ProCutiGen® Thermal Shield** to virgin hair followed by heat styling demonstrated a 34.3% decrease in protein loss when compared to the virgin hair alone. The hair samples treated with **ProCutiGen® Thermal Shield** had less protein loss, indicating that there was less damage to the hair cuticle. This data supports that by forming a *de novo* cuticle on the hair shaft, **ProCutiGen® Thermal Shield** is able to proactively protect the hair cuticle.

Tensile Strength Data

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Tradename: ProCutiGen® Thermal Shield

Code: 20828

CAS #: 69430-36-0

Test Request Form #: 3152

Lot #: NC170406-F

Test Performed:
Flexabrasion

Methods & Materials

This study was conducted by salon professionals using Sensationnel Bare & Natural Brazilian 100% Virgin Remi Unprocessed Human Hair (Hair Zone Moonachie, NJ). One swatch, left unaltered, was analyzed as the control. Two test swatches were treated and submitted for testing. One swatch was treated, spritzed with water, allowed to dry, curled holding for 10 seconds and released. The other test swatch was treated, spritzed with a 2.0% **ProCutiGen® Thermal Shield** solution and water, allowed to dry, curled holding for 10 seconds and released. The swatch treatment was designed to mimic everyday effects of curling the hair. It is important to note no additives or fixatives were used in the test solution. This was done intentionally in order to visually see clear results.

Gaston College Textile Technology Center was asked to perform Tensile Strength on ten (10) hair swatches provided by Active Concepts, LLC. Gaston College used an Instron 5966 to perform the test, using test method ASTM-D2256-10. This method specifies the test conditions for determining the tensile properties of hair using the single-strand method. The process determines the quality of the raw material and aides in controlling the quality of the end product. To determine tensile strength and elongation at break, specimens are clamped in the appropriate grips and extended at constant rate until failure occurs.

According to ASTM-D2256-10, single-strand hair specimens are broken on a tension testing machine at a predetermined elongation rate and the breaking force and the elongation at break are determined. Elongation at a specified force or the force or tenacity at a specified elongation may also be obtained. Breaking force, breaking tenacity, elongation, initial and chord modulus, and breaking toughness of the test specimen, in terms of linear density, may be calculated from machine scales, dials, recording charts, or by an interfaced computer.

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Results

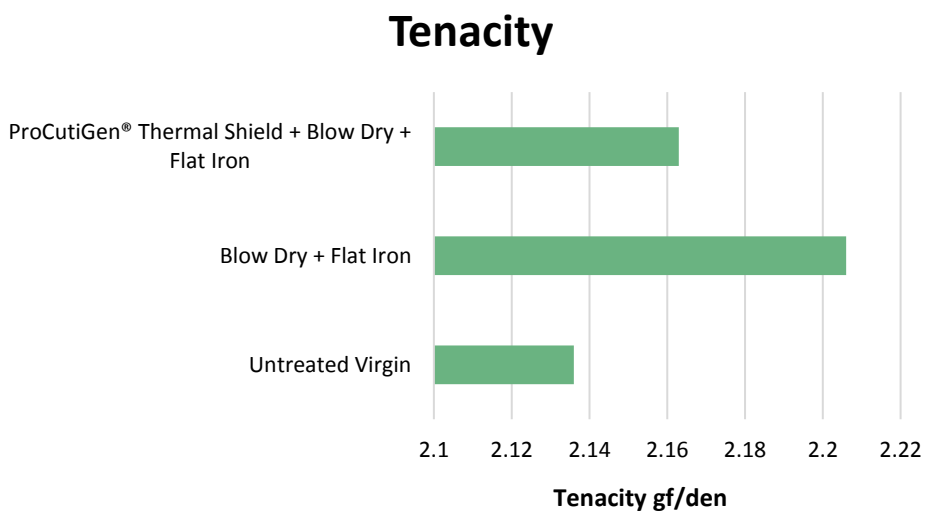

Figure 1. Tenacity, defined as the ultimate (breaking) force of the fiber (in gram-force units) divided by the denier

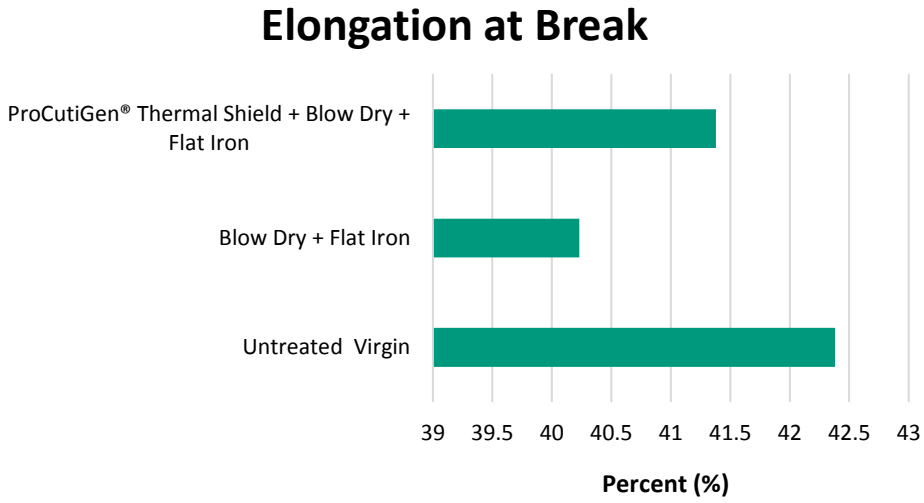

Figure 2. Elongation at break or fracture strain of test fibers

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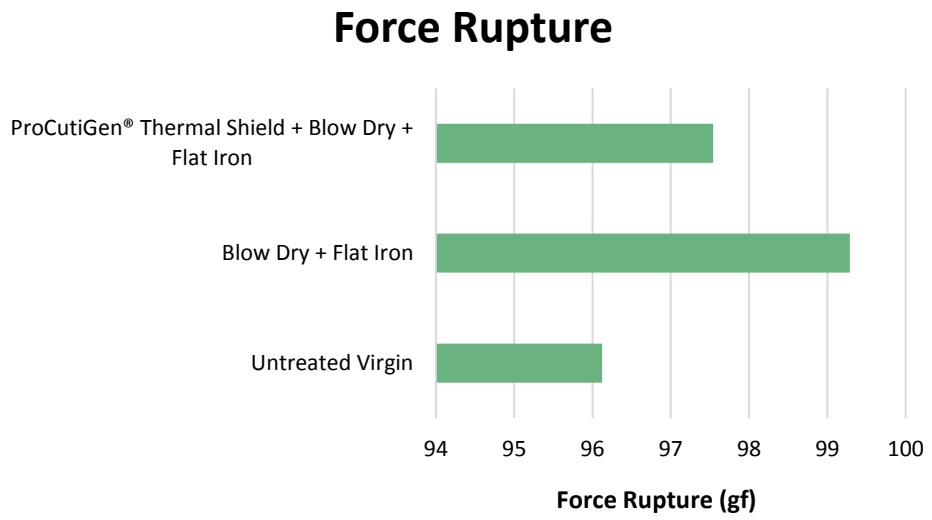

Figure 3. Force to rupture, is the force measured in gram-force (gf) necessary to rupture the hair

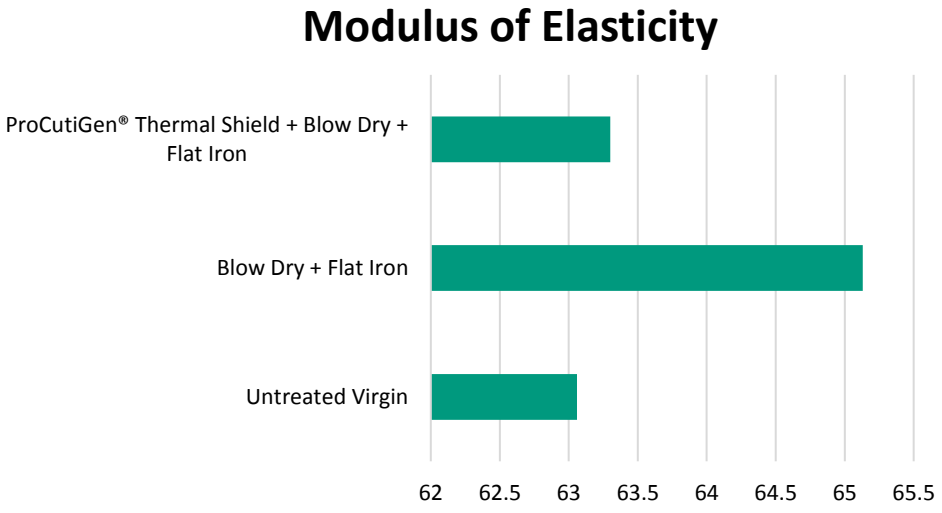

Figure 4. Modulus of Elasticity (Young's Modulus) is the ratio of tensile stress to tensile strain (gf/den)

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Tensile Strength Data

Discussion

Tensile strength is defined as the resistance of a material to break under tension. Gaston College Textile Technology Center assessed the following tensile strength factors; Tenacity, Elongation at Break, Force to Rupture, and Modulus of Elasticity (Young's).

Tenacity is the customary measure of strength of a fiber usually defined as the ultimate (breaking) force of the fiber (in gram-force units) divided by the denier. The results above indicate that **ProCutiGen® Thermal Shield** does not have an effect on this parameter.

Elongation at break, also known as fracture strain, is the ratio between changed length and initial length after breakage of the test specimen expressing the capability of a material to resist changes of shape without crack formation, how much a hair fiber will stretch before it breaks. Fibers that are weaker and less resistant to breakage have a greater elongation at break (%).The results above indicate that **ProCutiGen® Thermal Shield** does not have an effect on this parameter.

Force to rupture, is the force measured in gram-force (gf) necessary to rupture the hair. How long it takes to break the hair fiber. No significant improvement was exhibited using **ProCutiGen® Thermal Shield** on this parameter.

Modulus of Elasticity (Young's modulus) describes tensile elasticity, or the tendency of the hair to deform along an axis when opposing forces are applied along that axis; it is defined as the ratio of tensile stress to tensile strain (gf/den). The results above indicate that **ProCutiGen® Thermal Shield** does not have an pronounced effect on this parameter.

Parameters tested within this set of data are solely based on linear stress applied to the hair. Linear stress applied as a direct parallel force is not the ideal measure of real word stress and strain applied to the hair on a daily basis. In turn, **ProCutiGen® Thermal Shield** does not have an effect on this parameters nor do the claims associated with **ProCutiGen® Thermal Shield** relate to this testing.