Active Concepts

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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*
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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 450ºF 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 450ºF for 5 passes.

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3. **Virgin Water-Treated, Flat Ironed**
    a. Virgin Hair spritzed with water, blown dry for two minutes, and flat ironed at 450ºF for 5 passes.
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)

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Assay

1. Warm up spectrophotometer and blank with water.
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

A. Run Conditions:	100 Volts for 75 Minutes
B. Equipment:	Mini Gel Tank (Invitrogen); Power Supply (Hoefer); Pipettes
C. Gel:	10-well 14% Polyacrylamide
D. 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)
E. 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.

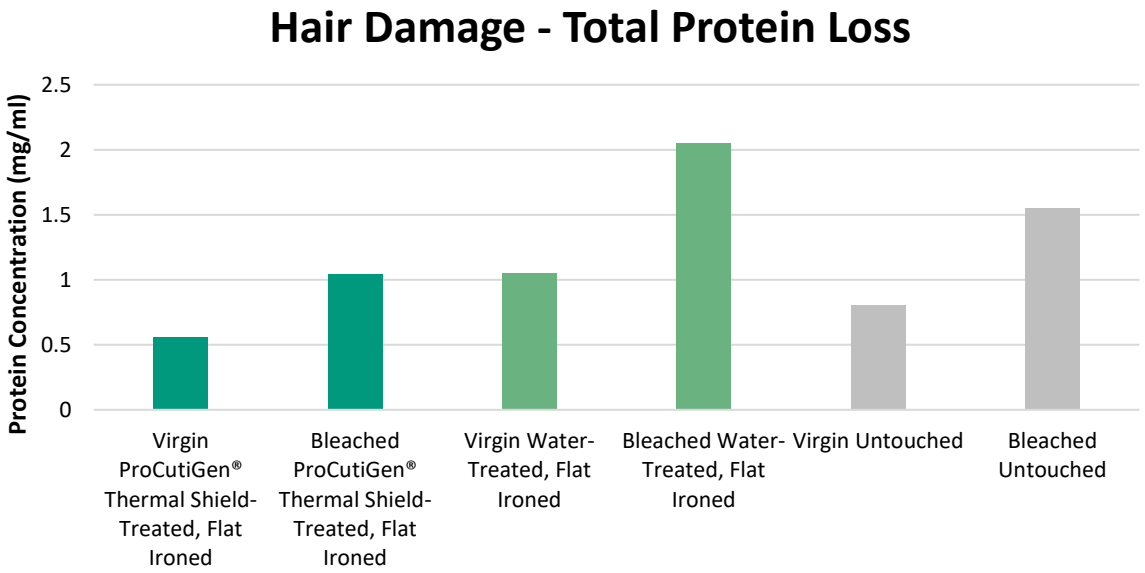

Figure 2. Concentration of extractable protein for each hair sample.

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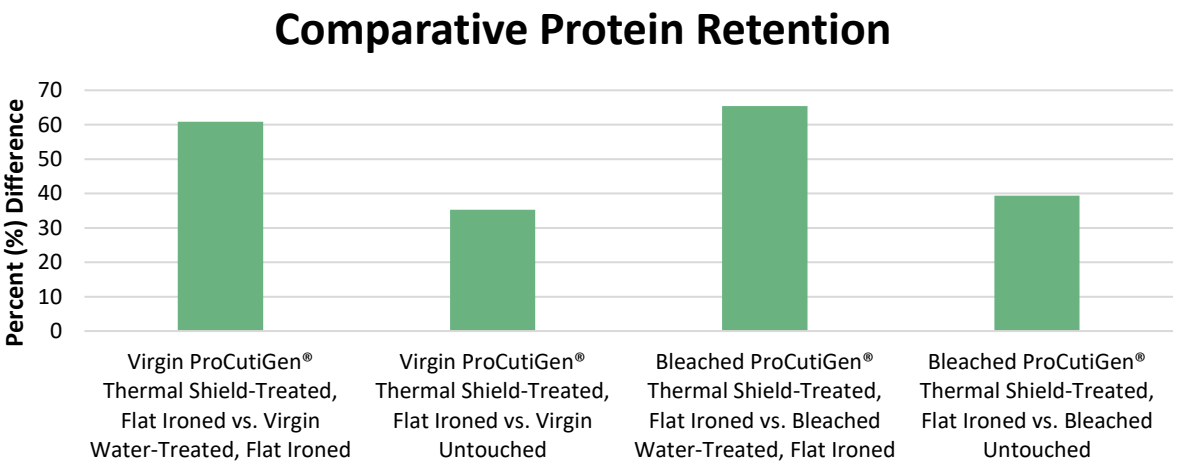

Figure 3. Comparative protein retention.

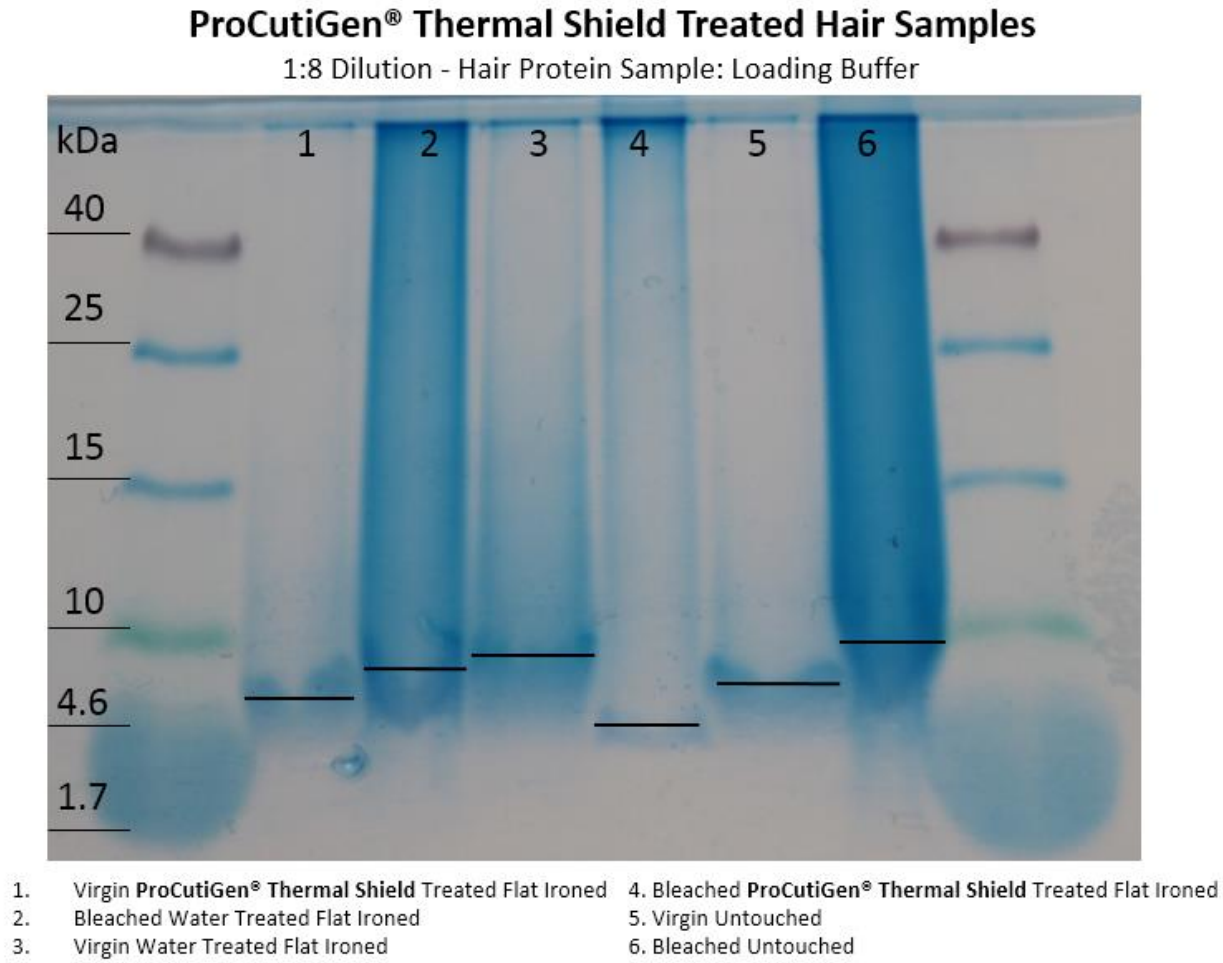

Figure 4. Protein gel electrophoresis of hair samples.

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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 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.