3D Hair Volume Assay

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Tradename: AC Volumizing Complex PF

Code: 16572PF

CAS #: 7732-18-5 & 65072-01-7 & 90027-90-0 & N/A

Test Request Form #: 12241

Lot #: 9403232

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Daniel Shill*
Principal Investigator: *Hannah Stade*

Test Performed:
3D Hair Volume Assay – Increasing Volume

Introduction
Hair density is genetically predetermined and defined as the number of hair follicles per given area on the scalp, whereas hair volume is the amount of space hair occupies. Hair volume can be altered with personal care products and is typically characterized by three metrics: amount of lift at the root, how full or large hair appears, and hair thickness. Consumers with fine or straight hair typically aim to add volume while those with curly or textured hair aim to control undesired volume. Therefore, cosmetic applications designed to alter hair volume are of particular importance.

Accordingly, a 3D Hair Volumizing Assay was performed to determine the hair volumizing properties of **AC Volumizing Complex PF**.

Assay Principle
Hair tresses are treated with various conditions and imaged with the 3D Bossa Nova Vision Rumba to determine changes in hair volume. The RUMBA software analyzes volume and hair alignment of tresses by quantifying the pixels occupied by the hair. Image analysis allows for both qualitative and quantitative analysis of hair volume before and after treatment.

Materials
A. Hair Samples:	Human Virgin Brunette Hair Tresses
B. Reagents & Products:	Base Shampoo and Conditioner (Table 1)
C. Equipment:	Bossa Nova Vision Rumba; Flat Iron (200 °C)
D. Software:	RUMBA; Excel Analysis ToolPak (Microsoft)

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Table 1. Base Shampoo and Base Conditioner Compositional Breakdowns.

Base Shampoo Formulation	
INCI	%
Water	41.0
Guar Hydroxypropyltrimonium Chloride	1.0
Sodium Methyl 2-Sulfolaurate (and) Disodium 2-Sulfolaurate	35.0
Cocamidopropyl Betaine	15.0
Lactobacillus Ferment & Lactobacillus & Cocos Nucifera (Coconut) Fruit Extract	4.0
Polysorbate 20	2.0
Fragrance	2.0

Base Conditioner Formulation	
INCI	%
Water	76.0
Polyquaternium-10	1.0
Glycerin	3.0
Water & Centrimonium Chloride	2.0
Behentrimonium Methosulfate & Cetearyl Alcohol & Butylene Glycol	8.0
Hydrogenated Ethylhexyl Olivate (and) Hydrogenated Olive Oil Unsaponifiables	5.0
Lactobacillus Ferment	4.0
Fragrance	1.0

Methods

Six hair tresses per condition were collected and utilized in this assay. Before treatment, all tresses were prewashed with sodium lauryl sulfate to ensure clean hair. After washing, hair tresses were treated with either Base Shampoo and Conditioner or 2.0% **AC Volumizing Complex PF** in Base Shampoo and Conditioner (Table 2). Tresses underwent six wash cycles as described below. First, tresses were dampened, then one mL of the respective shampoo was massaged into the hair for 30 seconds before rinsing for one minute. Next, each tress was treated for 30 seconds with one mL of conditioner before rinsing for one minute. Finally, tresses were allowed to fully air dry. A final seventh cycle was conducted where tresses received one mL of shampoo for one minute before rinsing for two minutes. After shampooing, one mL of conditioner was applied for one minute, rinsed out for two minutes, then allowed to fully air dry.

Table 2. Description of Treatment Conditions

Condition	Treatment Description
Base Shampoo and Conditioner	Base Shampoo and Conditioner
2.0% **AC Volumizing Complex PF**	2.0% **AC Volumizing Complex PF** in Base Shampoo and Conditioner

Tresses were analyzed before the treatment cycles and after the final air dry. Hair was straightened four times with a flat iron at 200 °C before analysis to ensure volume was not skewed by the hair curl pattern. Hair volume was quantified using the Bossa Nova Vision RUMBA which uses NIR LEDs to capture an intensity image of the hair. RUMBA software then determines hair volume and alignment by subtracting the background and analyzing pixel number and angle. Hair volume is evaluated as the integral between cursors where a higher value indicates an increase in hair volume. A color-coded fusion filter is applied to visualize hair orientation, and an alignment coefficient is calculated where a higher value indicates an increase in hair alignment.

Hair volume for each condition was averaged and data was analyzed using a one-way ANOVA with statistical significance accepted at $p \leq 0.05$. Percent change is expressed relative to baseline volume (before treatment) for each condition and calculated by the following equation:

$$Percent\ Change\ (\%) = \frac{Volume_{After\ Treatment} - Volume_{Baseline}}{Volume_{Baseline}} \times 100$$

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Results

The data obtained met criteria for a valid assay. Compared to the Base Shampoo and Conditioner, hair treated with **AC Volumizing Complex PF** demonstrated an increase in hair volume and improved alignment.

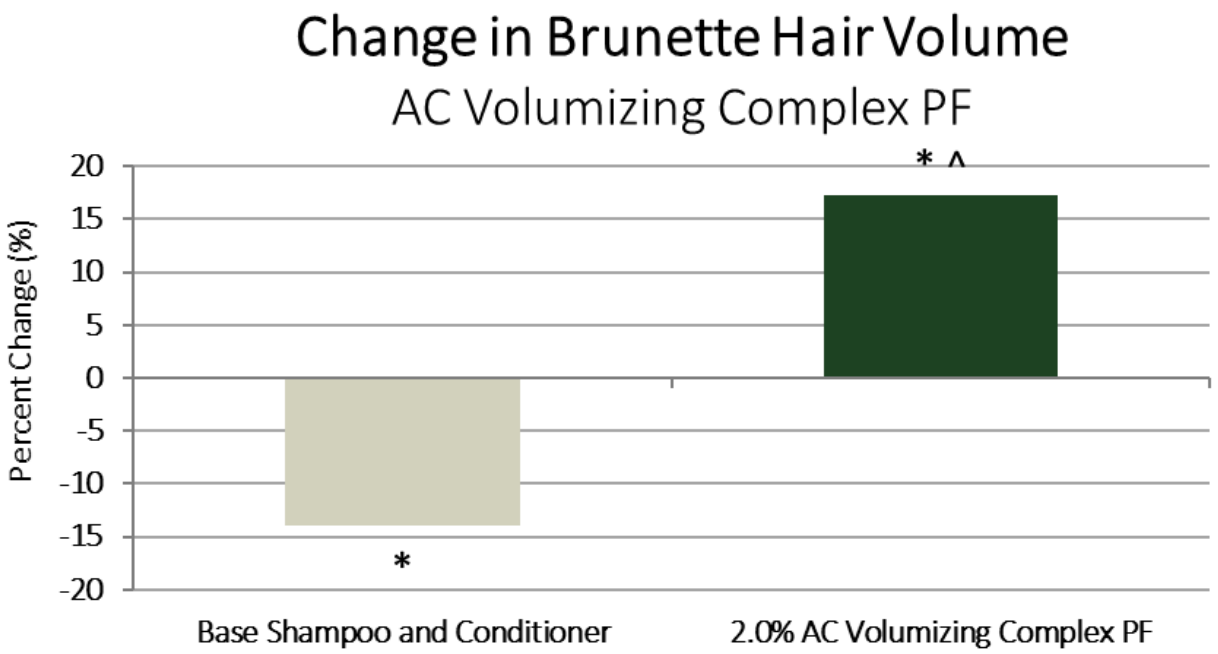

Figure 1. Percent Change in Hair Volume After Treatment Compared to Baseline. * indicates significance ($p \leq 0.05$) compared to Baseline within each condition. ^ indicates significance ($p \leq 0.05$) between conditions.

Table 3. Results from one-way ANOVA Statistical Analysis for Changes in Brunette Hair Volume within Conditions Compared to Baseline. * indicates significance ($p \leq 0.05$) compared to Baseline within each condition.

	Base Shampoo and Conditioner	2.0% **AC Volumizing Complex PF**
P-value	0.008*	0.030*

Table 4. P-values from one-way ANOVA Statistical Analysis for Brunette Hair Volume Between Conditions after Treatment. ^ indicates significance ($p \leq 0.05$) between conditions.

	Base Shampoo and Conditioner Vs 2.0% **AC Volumizing Complex PF**
P-value	0.026^

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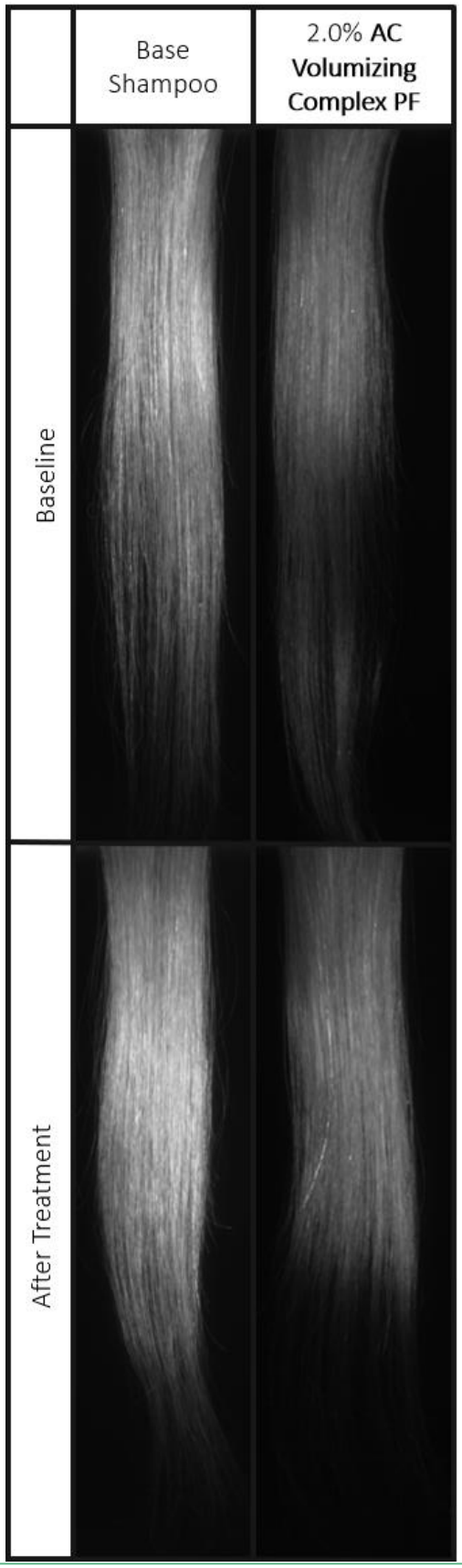

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Figure 2. Representative Intensity Images of Brunette Hair Tresses at Baseline and After Treatment.

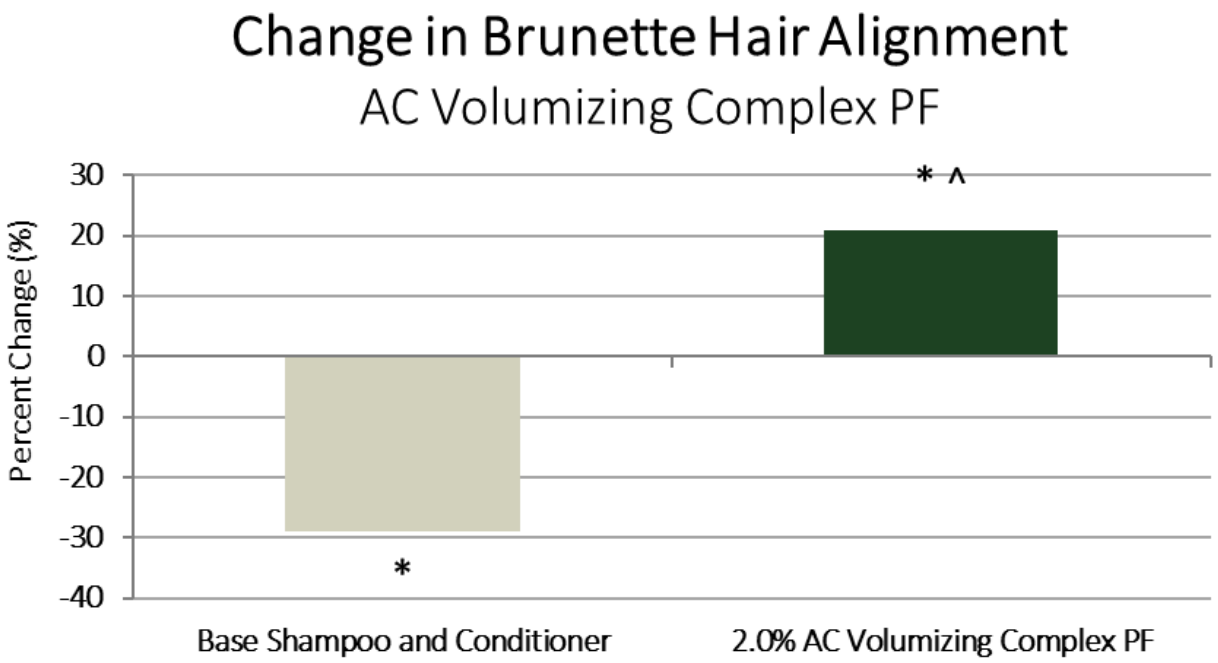

Figure 3. Percent Change in Hair Alignment After Treatment Compared to Baseline. * indicates significance ($p \leq 0.05$) compared to Baseline within each condition. ^ indicates significance ($p \leq 0.05$) between conditions.

Table 5. Results from one-way ANOVA Statistical Analysis for Changes in Brunette Hair Alignment within Conditions Compared to Baseline. * indicates significance ($p \leq 0.05$) compared to Baseline within each condition.

	Base Shampoo and Conditioner	2.0% **AC Volumizing Complex PF**
P-value	0.037*	0.004*

Table 6. P-values from one-way ANOVA Statistical Analysis for Brunette Hair Alignment Between Conditions after Treatment. ^ indicates significance ($p \leq 0.05$) between conditions.

	Base Shampoo and Conditioner Vs 2.0% **AC Volumizing Complex PF**
P-value	0.005^

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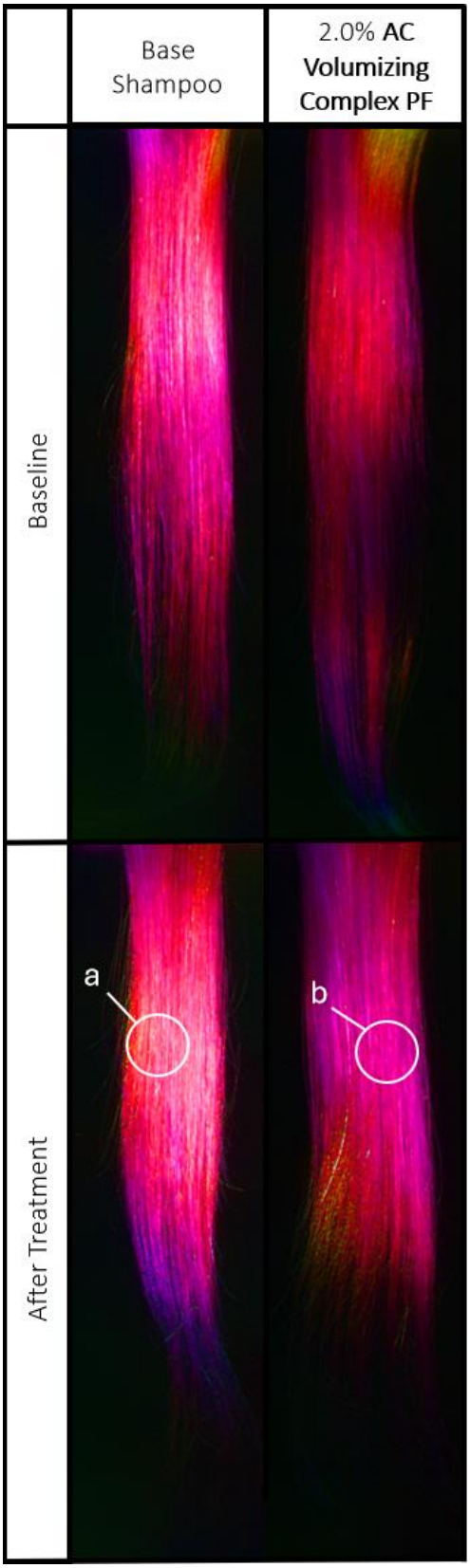

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Figure 4. Representative Fusion Images of Brunette Hair Tresses at Baseline and After Treatment. Mixed colors indicate more frizz (a), and solid colors indicate improved hair alignment (b).

Discussion

As shown in Figure 1, brunette hair strands treated with Base Shampoo and Conditioner exhibited a significant 14% decrease in volume compared to baseline (Table 3). Moreover, the tresses experienced a significant decrease of 29% in fiber alignment indicative of frizz (Figure 3, Table 5). Visibly, the tresses appear flat and frizzy compared to the baseline (Figures 2 and 4). Collectively, this data indicates the Base Shampoo and Conditioner negatively impacts brunette hair volume.

Alternatively, brunette tresses treated with 2.0% **AC Volumizing Complex PF** in Base Shampoo and Conditioner displayed a significant 17% increase in volume compared to baseline (Figure 1, Table 3). Additionally, the hair exhibited significantly improved alignment of 21% indicating frizz free fibers (Figure 3, Table 5). Visibly, the hair appeared fuller while maintaining a frizz free appearance compared to baseline (Figures 2 and 4). Moreover, a significant difference in volume and alignment is observed between the Base Shampoo and Conditioner and 2.0% **AC Volumizing Complex PF** after treatment (Tables 4 and 6). This data demonstrates the volumizing properties of **AC Volumizing Complex PF** on brunette hair.

Taken together, these results indicate **AC Volumizing Complex PF** augments hair volume and prevents frizz after repeated washing when added to shampoo and conditioner at recommended use levels. Collectively, **AC Volumizing Complex PF** demonstrates hair volumizing properties which contributes to the appearance of thicker healthy brunette hair.