

Tradename: AC Blue'Nette Toner

Code: 21027

CAS #: 999999-99-4 & 68333-16-4 (or) 92128-79-5 & 90082-41-0

Test Request Form #: 10483

Lot #: N230918G

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*

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Test Performed:

Brunette Hair Toning Assay

Introduction

Temporary hair dyes, such as blue- or purple-pigmented shampoos, act as a toner to brighten the original hair shade to a lighter and more vibrant color. Blue shampoos use ingredients that are designed to counteract orange tones in brunette hair, while purple shampoos are more often used to rid brass in lighter blonde shades. These hair care products typically use large blue or purple dye molecules to extrude brassy tones from hair and return it to a cooler and more salon-fresh shade. Consistent use of these pigmented shampoos aid bleached or color-treated hair to appear more vibrant. Specifically, these blue- and purple-pigmented shampoos camouflage dull yellow and orange tones in permanently dyed or bleached hair after prolonged exposure to tarnishing agents (sebum, sweat, pollutants, etc.). However, increased consumer demand for more natural alternatives has led to a gap in the formulation of these blue or purple shampoos that traditionally use textile acid dyes. To achieve a similar toning or brightening effect on hair historically obtained with pigmented shampoos, **AC Blue'Nette Toner** was designed as a natural and pigment-free alternative.

Accordingly, a qualitative and quantitative assay was performed to determine the *ex vivo* color toning ability of **AC Blue'Nette Toner** on virgin and partially bleached brunette hair.

Assay Principle

Brunette human hair tresses were partially bleached blonde or left virgin as these shades are prone to exhibiting orange tones. Tresses were imaged before and after treatment with test materials and analyzed for color shifts.

Materials

- A. Hair Sample:** Virgin Brunette Human Hair Tresses; Partially Bleached Brunette Human Hair Tresses
B. Product: Base Shampoo (Table 1), Redken Color Extend Brownlights™ Blue Shampoo
C. Software: ImageJ (NIH); Excel Analysis ToolPak (Microsoft)

Table 1. Base Shampoo Compositional Breakdown.

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

Methods

Images were taken before and after washing five virgin brunette hair tresses and five partially bleached brunette hair tresses with the following conditions: Untreated (no shampoo, water rinse), Base Shampoo, Redken Color Extend Brownlights™ Blue Shampoo, 2.0% **AC Blue'Nette Toner** in a Base Shampoo, and 5.0% **AC Blue'Nette Toner** in a Base Shampoo. The Redken Blue Shampoo was implemented for comparative purposes as a market standard, blue-pigmented shampoo. Each hair tress was evenly washed in its designated treatment and products were allowed to sit on the hair for four minutes. Tresses were then rinsed thoroughly and blown dry for one minute. ImageJ histogram analysis was performed on the acquired images to evaluate the red, green, and blue (R+G+B) color distribution present in each hair tress. The R+G+B color spectrum ranges from 0 (left) to 255 (right), where the left side of each histogram reflects exclusively red pixels, and the right side of each histogram reflects exclusively blue pixels. Percent change was calculated using the formula below:

$$\text{Percent Change (\%)} = \frac{\text{Mean}_{\text{Treatment Step}} - \text{Mean}_{\text{Before Treatment}}}{\text{Mean}_{\text{Before Treatment}}} \times 100$$

Three separate experiments were performed, and average values were recorded. Data was analyzed using a one-way ANOVA with statistical significance accepted at $p \leq 0.05$.

Results

The data obtained from this study met criteria for a valid assay and the controls performed as anticipated. **AC Blue'Nette Toner** in the base shampoo reduced orange tones in both virgin and partially bleached brunette hair.



Figure 1. Representative images of virgin brunette hair tresses before and after washing with corresponding R+G+B histograms. The green arrows indicate a shift towards the blue end of threshold indicating a reduction in orange tones.



Figure 2. Representative images of partially bleached brunette hair tresses before and after washing with corresponding R+G+B histograms. The green arrows indicate a shift towards the blue end of threshold indicating a reduction in orange tones.

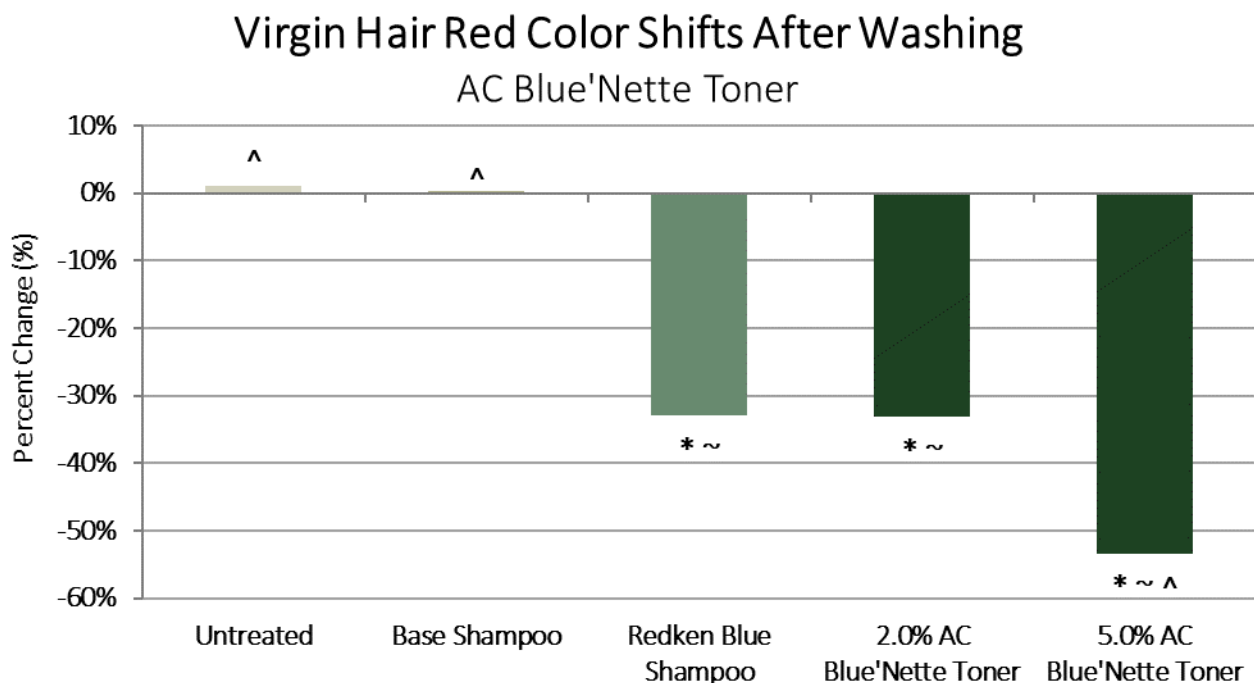


Figure 3. Virgin brunette hair shifts in red color (R+G+B intensity) after washing. A positive percent change indicates a shift towards more red tones (indicating an increase in orange tones), whereas a negative percent change indicates a shift towards bluer tones (indicating a reduction in orange tones). * indicates significance ($p \leq 0.05$) compared to Untreated. ~ indicates significance ($p \leq 0.05$) compared to Base Shampoo. ^ indicates significance ($p \leq 0.05$) compared to Redken Blue Shampoo.

Table 2. Results from one-way ANOVA between Conditions Compared for Virgin Brunette Hair Shifts in Red Color. * indicates significance ($p \leq 0.05$) compared to Untreated. ~ indicates significance ($p \leq 0.05$) compared to Base Shampoo. ^ indicates significance ($p \leq 0.05$) compared to Redken Blue Shampoo.

	Untreated	Base Shampoo	Redken Blue Shampoo	2.0% AC Blue'Nette Toner	5.0% AC Blue'Nette Toner
Untreated	-----	> 0.05	0.003*	0.004*	< 0.001*
Base Shampoo	> 0.05	-----	0.026~	0.033~	< 0.001~
Redken Blue Shampoo	0.003^	0.026^	-----	> 0.05	0.015^

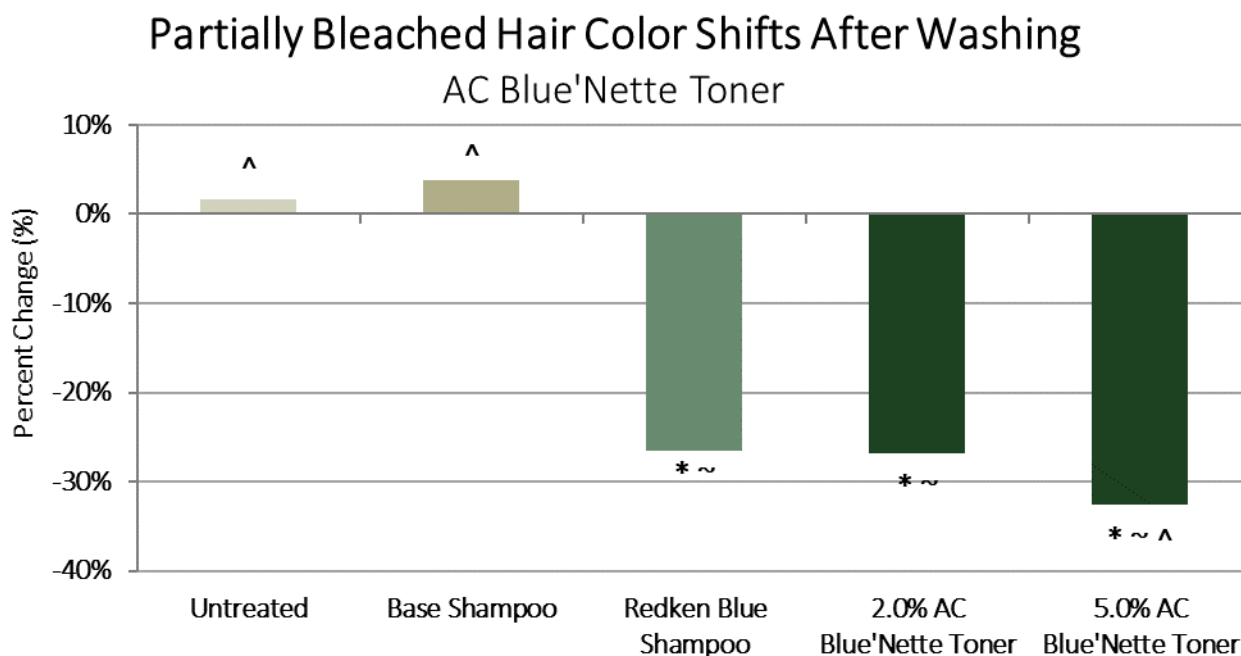


Figure 4. Partially bleached brunette hair shifts in red color (R+G+B intensity) after washing. A positive percent change indicates a shift towards more red tones (indicating an increase in orange tones) whereas a negative percent change indicates a shift towards bluer tones (indicating a reduction in orange tones). * indicates significance ($p \leq 0.05$) compared to Untreated. ~ indicates significance ($p \leq 0.05$) compared to Base Shampoo. ^ indicates significance ($p \leq 0.05$) compared to Redken Blue Shampoo.

Table 3. Results from one-way ANOVA between Conditions Compared for Partially Bleached Brunette Hair Shifts in Red Color. * indicates significance ($p \leq 0.05$) compared to Untreated. ~ indicates significance ($p \leq 0.05$) compared to Base Shampoo. ^ indicates significance ($p \leq 0.05$) compared to Redken Blue Shampoo.

	Untreated	Base Shampoo	Redken Blue Shampoo	2.0% AC Blue'Nette Toner	5.0% AC Blue'Nette Toner
Untreated	-----	> 0.05	0.004*	0.005*	< 0.001*
Base Shampoo	> 0.05	-----	0.011~	0.026~	0.001~
Redken Blue Shampoo	0.004^	0.011^	-----	> 0.05	0.044^

Discussion

As demonstrated in Figures 1 and 2, the untreated virgin and partially bleached brunette hair tresses visibly retained orange tones after washing. Quantitatively, R+G+B histogram analysis after washing without shampoo confirmed the presence of orange tones which increased slightly by 1% and 2% (shift towards the red side of the spectrum) for the virgin and partially bleached hair tresses, respectively, compared to before washing (Figures 1-4). Similarly, washing the virgin and partially bleached hair tresses with the base shampoo exhibited no changes and a 4% increase in orange tones, respectively, compared to before washing (Figures 1-4). This data demonstrates orange tones in both brunette hair tresses were not removed after washing with just water (no shampoo) and the base shampoo.

Alternatively, both hair tresses washed with Redken Blue Shampoo had visibly reduced orange tones (Figures 1 & 2). Histogram analysis confirmed the market standard, Redken Blue Shampoo significantly reduced orange tones by 33% and 27% in virgin and partially bleached hair tresses, respectively, compared to before washing (Figures 1-4, Tables 2 and 3). This data demonstrates market standard, blue-pigmented shampoos effectively reduce orange tones in virgin and partially bleached brunette hair.

The 2.0% and 5.0% **AC Blue'Nette Toner** shampoos visibly removed orange tones present in the virgin and partially bleached brunette hair tresses. Regarding histogram analysis, orange tones were significantly reduced by 33% and 53% in the virgin hair tresses washed with 2.0% and 5.0% **AC Blue'Nette Toner**, respectively, compared to before washing (Figures 1 and 3, Table 2). Similarly, the partially bleached hair tresses exhibited significant 27% and 33% decreases in orange tones after washing with 2.0% and 5.0% **AC Blue'Nette Toner**, respectively, compared to before washing (Figures 2 & 4, Table 3). This data demonstrates **AC Blue'Nette Toner** achieves a comparable toning effect in virgin and partially bleached brunette hair to a market standard, blue-pigmented shampoo.

Collectively, these results indicate **AC Blue'Nette Toner** achieves a similar qualitative and quantitative toning effect on virgin and partially bleached brunette hair to a market standard, blue-pigmented shampoo when utilized at the recommended use-levels. In summary, **AC Blue'Nette Toner** extrudes orange tones from brunette hair and is a natural, pigment-free color toning alternative to products with large and acidic dye molecules.