

BiEau® Actif Tri-Mushroom



sustainable Antioxidant
Nourishing
anti-inflammatory isotonic solution
cellular plant essence Conditioning

BACKGROUND

Plants naturally utilize and bind water in their cells as a mechanism of survival. This cellular water or plant essence offers the ability to harness a nutritional, isotonic solution capable of nourishing the skin and hair in personal care applications. BiEau® Actif botanical waters combine advancements in sustainable beauty and leading edge science to provide brands essential ingredients to support their unique aspirations. Innovative development of botanical waters promote the natural balance of the skin, scalp, and hair while utilizing natural plant properties to work in conjunction with the skin. Botanical waters offer brand-differentiating properties including unique origin stories, sustainability appreciation, and efficacious beauty benefits that work with your hair and skin care for a healthy complexion. From cultured algal and mushroom cells to reanimated agricultural waste, Active Concepts maximizes raw material sourcing and creation for minimal environmental impact with the development of our BiEau® Actif botanical waters line.

BiEau® Actif Tri-Mushroom is a vital botanical essence sustainably sourced from *Ganoderma lucidum*, *Inonotus obliquus*, and *Cordyceps sinensis*, designed to encourage an isotonic environment while reducing inflammation and promoting antioxidant protection for the skin. *Ganoderma lucidum*, *Inonotus obliquus*, and *Cordyceps sinensis*, are three mushroom species commonly utilized to optimize skin health and are recognized as an abundant source of nutrients including glutamine. In the body, glutamine downregulates inflammatory pathways, diminishes oxidative stress, and acts as a precursor for antioxidant molecules such as glutathione. **BiEau® Actif Tri-Mushroom** utilizes a novel approach to sustainability while capitalizing on the presence of glutamine, from three mushroom species, to rejuvenate the complexion with potent anti-inflammatory and antioxidant benefits.

BiEau® Actif Tri-Mushroom utilizes cellular plant essence to deliver isotonic nutrients and harmonize skin and hair. Skin is the largest organ of the body and is composed of approximately 60% water. As a protective barrier, the skin is our best defense against external aggressors. However, the skin's permeability can

Code Number: 16908

INCI Name: Ganoderma Lucidum Extract & Inonotus Obliquus (Mushroom) Extract & Cordyceps Sinensis Extract

INCI Status: Proposed

REACH Status: Complies

CAS Number: 223751-82-4 & 2055734-24-0 & 1174745-80-2

EINECS Number: 607-059-7 & N/A & N/A

Origin: Botanical

Processing:

- GMO Free
- No Ethoxylation
- No Irradiation
- No Sulphonation

Additives:

- Natural Antimicrobial: Lactobacillus Ferment
- Preservatives: None
- Antioxidants: None
- Other additives: None

Solvents Used: N/A

Appearance: Slightly Hazy to Hazy Liquid

Soluble/ Miscible: Water Soluble
95.9% Biodegradability

Microbial Count: <100 CFU/g,
No Pathogens

Suggested Use Levels: 1.0 -10.0%

Suggested Applications:

Benefits of BiEau® Actif Tri-Mushroom:

- Antioxidant
- Isotonic Solution
- Anti-Inflammatory



BiEau® Actif Tri-Mushroom

be influenced by a variety of environmental attributes including pollution particles, cosmetic actives, temperature fluctuations, and water exchange. A delicate balance exists between water introduced onto our skin, using cosmetics, cleansers etc, and the water present in our skin and cells. Water exchange occurs through the cell membrane through the process of osmosis. Deionized or demineralized water can be traditionally used in the creation of cosmetic ingredients, but this water typically lacks minerals and natural components that our skin craves. Isotonic solutions offer an environment most beneficial and least disruptive to skin cells while enhancing affinity with the skin due to a mineral content close to that of skin cells. Isotonic materials maintain the size, shape, and integrity of skin cells. Some cosmetic solutions are hypotonic or hypertonic and can damage skin cells by drawing out cellular water, thereby drying out the skin, or even forcing water into the cells and causing cell deformation. Isotonic solutions maintain a natural environment and healthy balance for the skin and hair.

SCIENCE

Hailed as healthy for the planet, mushrooms maintain a low carbon footprint and a sustainable life cycle. Packed with protein, fiber, vitamins, and minerals, mushrooms promote healthy beauty inside and out. Glutamine is an amino acid present in mushrooms that lends to its ability to provide potent antioxidant and anti-inflammatory benefits in beauty products to encourage a rejuvenated aesthetic. Grown in house, cultured mushroom cells diminish the demand for mushroom harvesting while allowing the manufacture of various ingredients including mushroom botanical water. The renewable nature of mushrooms prompt their incorporation into beauty applications across skin, hair, and body care formulations.

Mushrooms are approximately 90% water and are able to absorb and hold water from their surroundings. This internal cellular water typically resides in the vacuoles, cytoplasm, and cell walls of the mushrooms and contains an abundance of biologically active components. Active Concepts has utilized solar energy to effectively evaporate and isolate the nutrient-rich water of *Ganoderma lucidum* and *Inonotus obliquus* and *Cordyceps sinensis* to produce a bioactive cellular water capable of promoting antioxidant protection and anti-inflammatory properties.

Glutamine is an amino acid present in a variety of foods and in our bodies. Mushrooms are a rich, natural source of glutamine. One of the unique benefits of glutamine as a skin care ingredient is its ability to promote antioxidant and anti-inflammatory benefits. Glutamine is involved in many metabolic activities including glutathione and protein synthesis as well as energy production. In the body, glutamine downregulates inflammatory pathways, diminishes oxidative stress, and acts as a precursor for antioxidant molecules such as glutathione. Antioxidants are powerful free radical scavengers working to protect and reduce potential damage to cells. Reactive oxygen species, ROS, can modify DNA resulting in cellular damage and in regards to skin care contribute to fine lines, wrinkles, and accelerated signs of aging. Inflammation is another driving force behind the skin aging process, leading to a breakdown of the extracellular matrix and consequential skin wrinkling. Downregulation of inflammatory pathways can alleviate inflammation damage including collagen and elastin degradation, as well as a breakdown of the skin's barrier function.

BENEFITS

BiEau® Actif Tri-Mushroom is a vital botanical essence capable of decreasing inflammation while offering antioxidant protection. **BiEau® Actif Tri-Mushroom** utilizes a novel approach to eco-conscious cosmetic active development while capitalizing on the presence of glutamine from three mushroom varieties, including *Ganoderma lucidum* and *Inonotus obliquus* and *Cordyceps sinensis*, that work together to promote a healthy complexion. **BiEau® Actif Tri-Mushroom** can be used in a variety of cosmetic and personal care formulations aimed to nourish and rejuvenate while encouraging an isotonic environment beneficial for optimal health of the skin and hair.

EFFICACY

An *in-vitro*, interleukin-6 ELISA assay was conducted to assess the changes in IL-6 levels in cultured human dermal fibroblasts treated with **BiEau® Actif Tri-Mushroom**. Interleukin-6 is a proinflammatory cytokine known to play an active role in inflammation, immunology, bone metabolism, reproduction, arthritis, neoplasia, and aging. IL-6 signals through the nuclear factor-kappa B pathway that results in the transcription of inflammatory mediators, including matrix metalloproteinase-1 (MMP-1). MMP's are responsible for breaking down the extracellular matrix and collagen in the skin leading to wrinkles, fine lines, and loss of skin elasticity. Reducing the level of IL-6 and other

BiEau® Actif Tri-Mushroom

inflammatory mediators is believed to slow down degradation of the skin matrix and, possibly, stimulate its replenishment. As shown in Figure 1, results indicate **BiEau® Actif Tri-Mushroom** exhibited anti-inflammatory effects on LPS-treated fibroblasts utilizing various concentrations of **BiEau® Actif Tri-Mushroom** including 1%, 0.1%, 0.01%. This decrease in IL-6 production indicates a reduced inflammatory environment, which could decrease the signs of aging and reduce the formation of fine lines and wrinkles. This study indicates, at normal use concentrations, **BiEau® Actif Tri-Mushroom** enhances soothing and anti-aging properties.

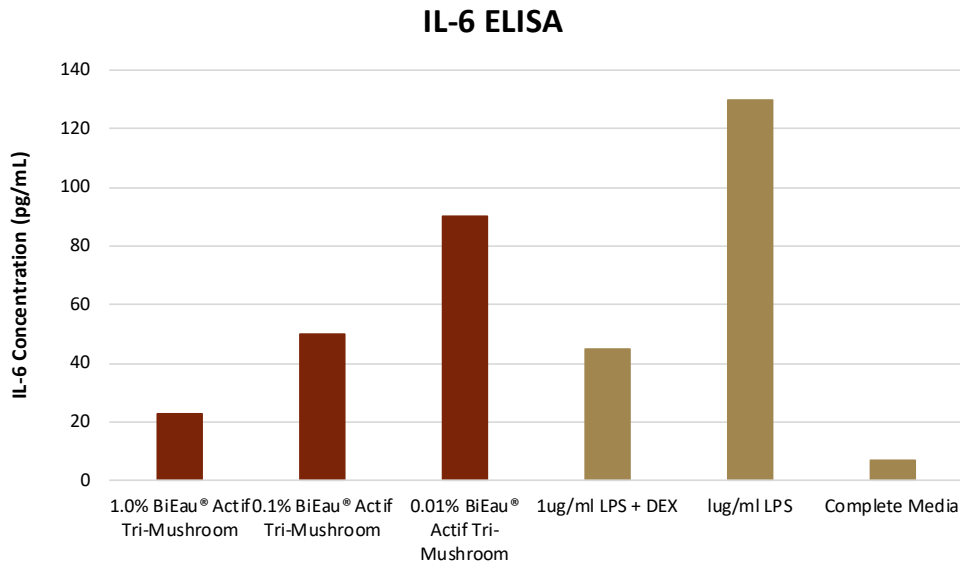


Figure 1. Anti-Inflamatory capacity of BiEau® Actif Tri-Mushroom

Furthermore, an *in-vitro* Oxygen Radical Absorbance Capacity (ORAC) assay was conducted to assess the antioxidant capacity of **BiEau® Actif Tri-Mushroom**. Reactive oxygen species (ROS) are generated by normal cellular processes, environmental stresses, and UV irradiation. ROS are dangerous to cellular structures and functional molecules (i.e DNA, proteins, lipids) as they act as strong oxidizing agents or free radicals. The ORAC assay is a standard method used to assess antioxidant capacity of physiological fluids, foods, beverages, and natural products. The assay quantitatively measures a sample's ability to quench free radicals that have the potential to react with and damage cellular components.

Figure 2 displays the ability of **BiEau® Actif Tri-Mushroom** to exhibit greater antioxidant activity than 200µM Trolox®. The antioxidant capacity of **BiEau® Actif Tri-Mushroom** increased as the concentration increased, therefore indicating that the ability to minimize oxidative stress is dose dependent. This study concludes that **BiEau® Actif Tri-Mushroom** is capable of providing antioxidant properties and aids in the anti-aging process offering protection at the cellular level.

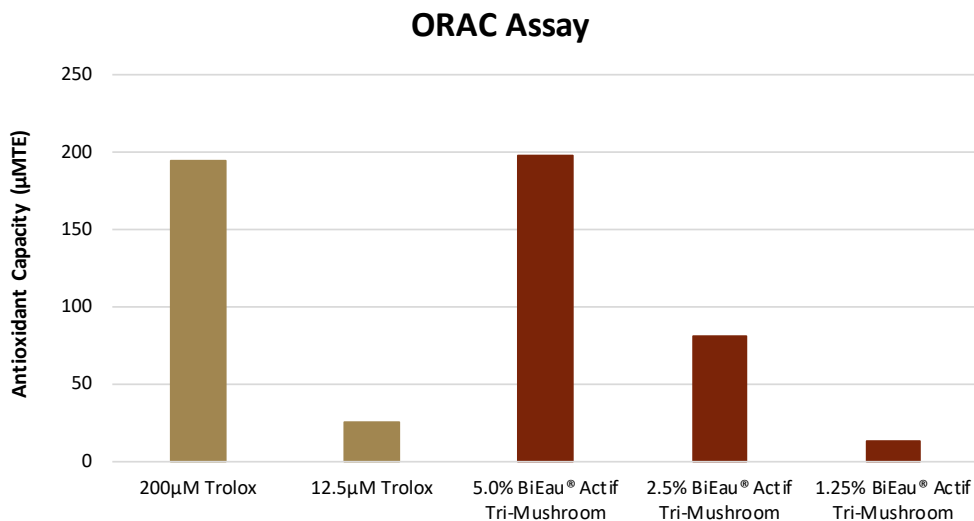


Figure 2. Antioxidant capacity of BiEau® Actif Tri-Mushroom

BiEau® Actif Tri-Mushroom

An Osmotic Cell Pressure Membrane study was performed to evaluate how cellular water or plant essence offers the ability to harness a nutritional, isotonic solution capable of nourishing the skin and hair in personal care applications. Human dermal keratinocytes were seeded into 24-well tissue culture plates and allowed to grow to confluency in complete media. The complete media was removed and either 100% Deionized Water or 100% **BiEau® Actif Tri-Mushroom** was added to the respective test well. Set to a 40x magnification, images were taken at time zero and every 5 minutes all the way up to the final 45 minute time mark. Crystal Violet Stain was used for enhanced microscopy imaging and a final stain image was taken of the test well.

As demonstrated in Figure 3, nature constantly tends to maintain balance. In the presence of a hypotonic deionized water solution, the deionized water will penetrate the skin cells to balance out the differences in concentration on either side of the membrane. Skin cells then swell due to the water pressure on the cell walls. The internal cellular water of the plants used in the production of our **BiEau® Actif** product line harnesses a nutritional, isotonic solution capable of supporting the skin's natural environment and promotes cellular balance. The skin cells remain in a natural environment and their morphology and integrity remain unaltered.

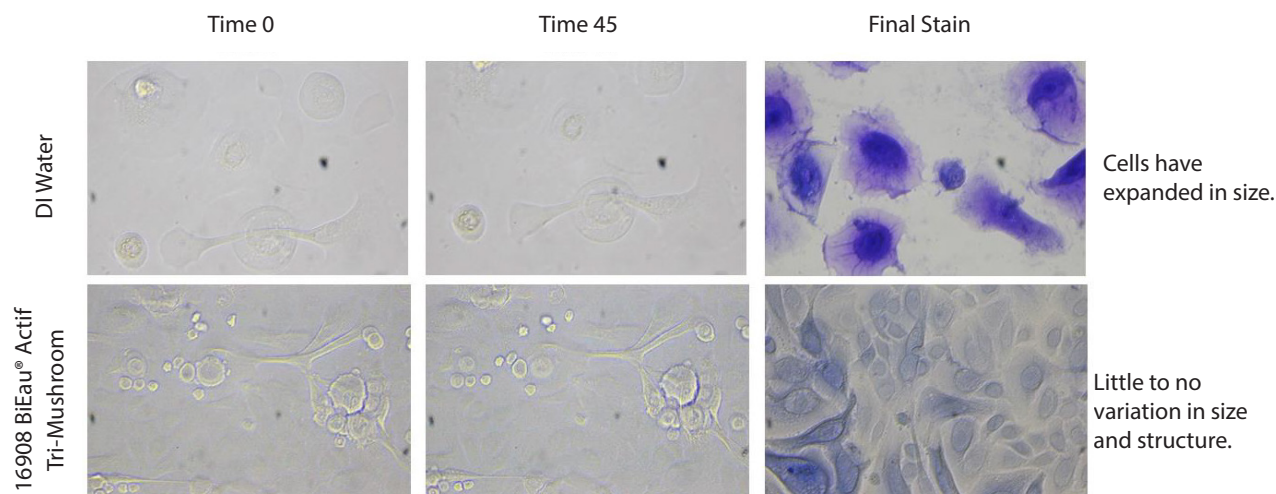


Figure 3. Cell Images.

*Please note that due to the post treatment fixation and staining, the plate was removed from the microscope to complete the process. Final stain images were taken of the exact same treatment culture well, though the individual cells captured may vary from the original images.

References:

1. Paudel, Ekaraj, et al. "Effects of cellular structure and cell wall components on water holding capacity of mushrooms." *Journal of food engineering* 187 (2016): 106-113.
2. Sabatino, A., Antoniotti, R. and Fiaccadori, E. (2019). *Critical Care Nephrology*. 3rd ed. Elsevier, p.Chapter 78 Management of Nutrition in Acute Kidney Injury and Renal Replacement Therapy.
3. Yamaguchi, Yu, et al. "Antioxidant activity of the extracts from fruiting bodies of cultured *Cordyceps sinensis*." *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives* 14.8 (2000): 647-649.
4. Zhang, J., Suk Ahn, W., Gameiro, P., Keibler, M., Zhang, Z. and Stephanopoulos, G. (2014). *Methods in Enzymology*. Elsevier, p.Chapter Nineteen 13C Isotope-Assisted Methods for Quantifying Glutamine Metabolism in Cancer Cells.