20793PF.

AC CytoSulf PF

BOTANICAL EXTRACTS

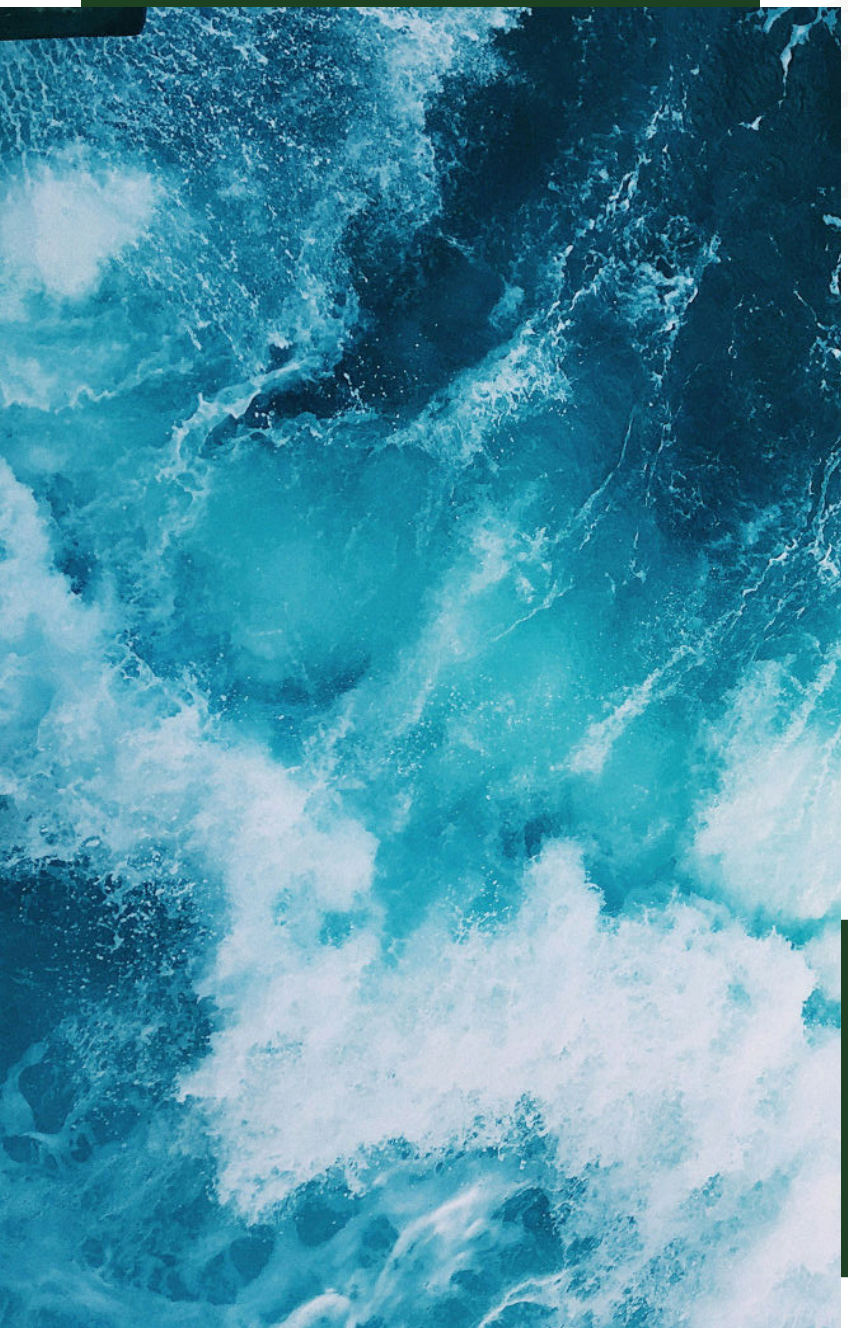
Active Concepts

VEGAN

IN VIVO

IN VITRO

CHINA



THE FEATURES.

AC CytoSulf PF is the cutting edge of age defying beauty. The induction of cellular stasis through plankton-derived sulfide donors is the perfect solution for permanently youthful skin. Without inducing cellular death, or overstimulating cell production, AC CytoSulf PF is capable of suspending cells in a semi-permanent, drawn out stage of rest. By utilizing phytoplankton's many antioxidant and anti-aging properties, Active Concepts offers a product capable of improving collagen production and moisture retention. For skin that defies aging through practical and understood science, AC CytoSulf PF is the solution.

Plankton Extract

Actions

Anti-Aging
Collagen Production
Moisture Retention
Antioxidant

TECHNICAL DATA SHEET.

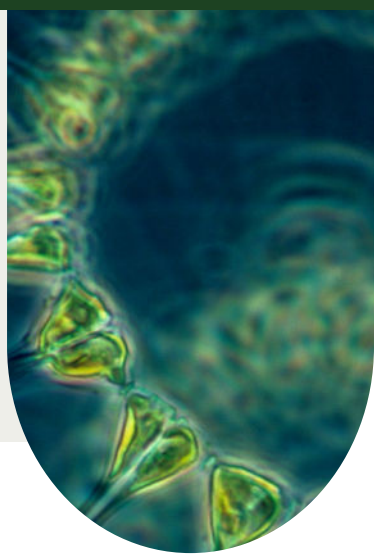
AC CytoSulf PF

THE REGULATION.

INCI. Plankton Extract
CAS. 91079-57-1
EINECS. 293-445-7
EUROPE. Compliant
USA. Exempt
CHINA. Compliant

THE SPECIFICATION.

Origin. Botanical/Bacteria
Natural Antimicrobial. Leuconostoc/Radish Root Ferment Filtrate*
Preservatives. None
Solvents Used. None
Soluble/Miscible. Water Soluble
Appearance. Clear to Slightly Hazy Liquid
Use Level. 1- 5 %



** Please note this product contains Leuconostoc/Radish Root Ferment Filtrate (Tradename: M15008-Leucidal® Liquid) - produced by Active Micro Technologies, LLC - containing 18.0–22.0% Phenolics (tested as Salicylic Acid). Please refer Leucidal® Liquid product literature for additional information.*

THE STORY.

How we experience the passage of time is the essence of aging. Our cells age according to a clock described by biologists as the cell cycle. The passage from one phase to the next marks the progression of the lifespan of a cell. The slower the cell moves through this cycle, the slower it ages and reaches its eventual senescence. Studies have confirmed that certain organisms living in extreme conditions can become quiescent for extended periods of time, an ability that humans lack. It's theorized that the quiescence seen by these certain species of extremophiles may be linked to cell cycle interruptions via sulfur-rich enzymes.[1] The discovery of a new class of gasotransmitters has allowed scientists to achieve what was once solely the realm of science fiction – the suspension of the aging process.

The intersection of these groundbreaking discoveries coupled with our expertise in bacterial fermentation has allowed us to capture specific sulfide donors from extreme prokaryotes such as *Sulfolobales*. These sulfide-rich peptides in topical applications are able to slow the cellular aging process and deliver unique pro-aging benefits. For example, the Hayflick limit, or the number of times a cell can divide before its telomeres become too short for replication, is an excellent marker of aging. By prolonging a cell's lifecycle, you are effectively delaying aging which could result in perceivably less wrinkles and more taught, supple, or even softer skin.

Furthermore, plankton-derived ingredients can be used in cosmetic products to maintain skin moisture, increase skin elasticity, and provide detoxification. Incorporating plankton-derived ingredients into hair care products can also strengthen hair follicles and impart shine.[2] As a natural cleanser or detoxifier, plankton can remove unwanted product or buildup from the scalp and hair without overdrying. This property can reduce damage, prevent further damage, and repair split ends while hydrating and conditioning the scalp too.

THE SCIENCE.

Current heart and lung tissue research focused on internal gasotransmitters (small gaseous molecules capable of signaling cells to induce both physical or chemical changes) suggests that sulfide donors can reduce damage in these tissues by decreasing the rate of cell cycle transition, specifically via sulfur dioxide (SO_2). Internally, SO_2 plays a key role in the crosstalk and regulation between pathways involved in cell stasis, such as cAMP/PKA (cyclic AMP Pathway/protein kinase A) and Erk/MAPK (extra cellular signal-related kinase/ mitogen activated protein kinase).[3,4]

Additionally, research on internal gastrotransmitters found that SO_2 inhibited vascular smooth muscle cell division by preventing cell cycle progression from G1 to S phase, and by DNA synthesis. Also important to note, findings supported that internal SO_2 did not influence vascular smooth muscle cell death in any way. Reducing it suspended the tissue in a type of stasis via suppression and control of the Erk/MAPK pathway mediated by cAMP/PKA signaling without causing cell death.[3] As with the endogenous gasotransmitter, SO_2, the FUCCI (fluorescence ubiquitination cell cycle indicator) cell cycle assay proves that this product can also halt the cell cycle of human skin cells, specifically in both the G2-M and G1 phase compared to the untreated controls. It is suspected that the oxidized sulfur from thermophilic cells act in the same fashion as internal SO_2, suspending human keratinocytes in a state of cytostasis.

By prolonging the cell cycle and reducing the rate of cell division, the potential for a new pathway and approach to anti-aging is here! One of the initial problems with utilizing sulfur as a method for inducing cell stasis is its assumed toxicity. By harnessing sulfide donors from *Chlorobium tepidum*, we have successfully eliminated the characteristic odor and cytotoxicity associated with sulfur-based cosmetics. Using the formulaic components of elemental sulfur (sulfide donors), naturally derived from *Chlorobium tepidum*, AC CytoSulf PF was engineered to increase cellular cytostasis while remaining procedurally simplistic to formulate with.

AC CytoSulf PF

THE BENEFITS.

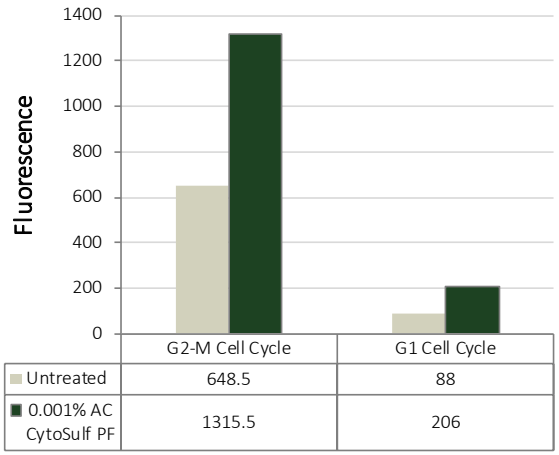
Skin

Anti-Aging	*Cytostasis Assay*
Antioxidant	*ORAC Assay*
Improved Collagen Density	*High-Resolution Ultrasound Skin Imaging Study*
Barrier Function	*Transepidermal Water Loss (TEWL) Study*
Soothing & Anti-Aging	*IL-6 ELISA Analysis*
Moisturizing	*Moisturization/ Hydration Assay*
Healing & Cell Proliferation	*Scratch Assay Analysis*

THE EFFICACY.

Cytostasis Assay.

A Cytostasis Assay was conducted to assess the *in vitro* ability of AC CytoSulf PF to induce cytostasis in human keratinocytes. Keratinocytes were seeded into 24-well tissue culture plates and allowed to grow to confluency in complete media. When confluency was reached, the cells were treated with 0.001% AC CytoSulf PF for 24 hours at 37°C, 5% CO_2, and 95% RH. Cells incubated in complete serum-free media were used as the control. A cell count was obtained from an untreated seeded well. This cell count was used to calculate the volume of two reagents, Premo™ geminin-GFP and Premo™ Cdt-1-RFP. After 24-hour incubation, the culture media containing the Premo™ geminin-GFP and Premo™ Cdt1-RFP reagents was removed, the wells were washed with complete serum-free media and fresh complete serum-free media was added. Excitation and emission wavelengths of 488/555 nm and 510/584 nm were utilized to assess the G2-M and G1 phases of the cell cycle, respectively. Phosphate Buffered Saline (PBS) was used to assess background fluorescence of the tissue culture plate. The background was subtracted from the fluorescence reading observed from the experimental wells. Results indicate that AC CytoSulf PF is capable of triggering cytostasis which may attenuate or reverse the visible alterations in skin structure that



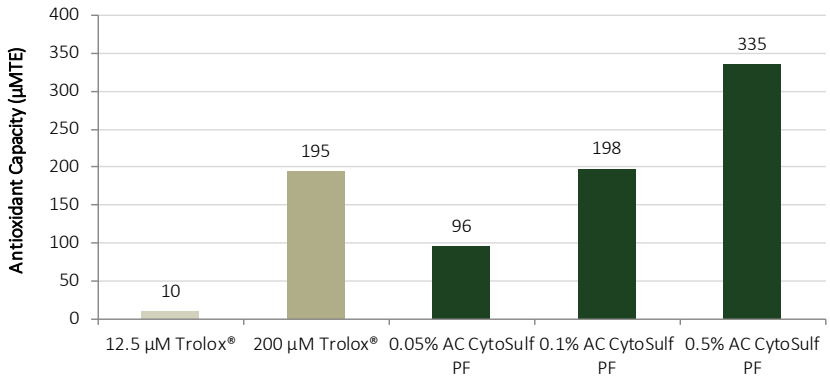
Induced a 68% to 80% increase in cytostasis in the G2-M & G1 cell cycles, respectively **(Tested at 0.001%)**

Anti-Aging.

Attenuate or reverse the visible alterations in skin structure

ORAC Assay.

The Oxygen Radical Absorbance Capacity (ORAC) Assay was conducted to assess the antioxidant capacity of AC CytoSulf PF. Solutions of AC CytoSulf PF and Trolox® (positive control) were prepared in 75 mM potassium phosphate buffer. Materials were prepared at three different concentrations/dilutions. Trolox® was used as a reference for antioxidant capacity and prepared at concentrations ranging from 12.5 µM to 200 µM in 75 mM potassium phosphate buffer. Results indicate that AC CytoSulf PF is capable of providing antioxidant properties and aids in the anti-aging process through protection at the cellular level.



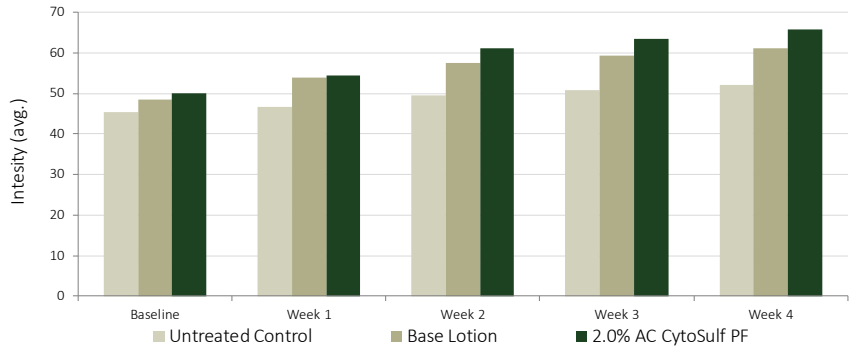
Increased antioxidant capacity by 53% compared to 200 µM Trolox® **(at 0.5%)**

Antioxidant.

Free radical quenching abilities & Cellular protection

High-Resolution Ultrasound Skin Imaging Study.

An *in vivo* High-Resolution Ultrasound Skin Imaging study was conducted over a period of four weeks to assess the ability of AC CytoSulf PF to improve collagen fiber density. High Resolution Ultrasound Skin Imaging is based on measuring the acoustic response after an acoustic pulse is sent into the skin. 10 volunteers between the ages of 23 and 45, and who were known to be free of any skin pathologies, participated in this study. Results indicate that AC CytoSulf PF is capable of improving collagen fiber density when compared to both the untreated control as well as the base lotion, meaning that this ingredient has a strong positive effect on skin density when used at recommended use level.



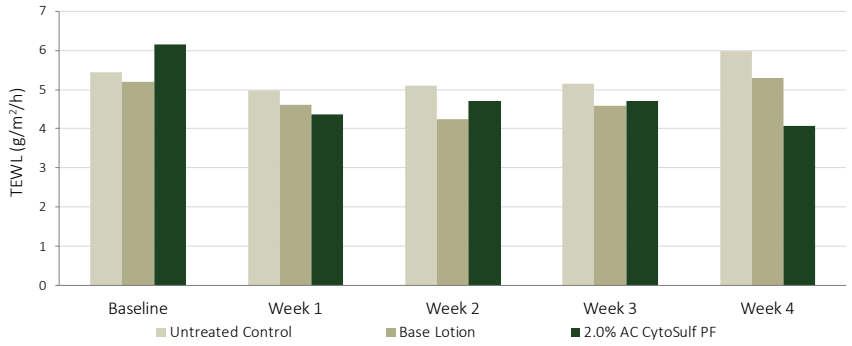
Improved skin density by 31% after 4 weeks compared to base lotion **(Tested at 2%)**

Skin Density.

Improves Skin Elasticity

Transepidermal Water Loss (TEWL) Study.

A Transepidermal Water Loss Assay was conducted to assess the moisture retention capabilities of AC CytoSulf PF. 10 volunteers who were known to be free of any skin pathologies participated in this study. TEWL measurements are made by placing a probe on the skin of pre-identified test sites. Following initial measurements, all subjects were asked to apply 0.2 g of each test material on their volar forearms twice a day for a four-week period. Measurements were taken once a week over the course of four weeks. The test material consisted of 2.0% AC CytoSulf PF in a base lotion. Results indicate that AC CytoSulf PF is capable of providing moisture retention benefits when added to personal care applications at recommended use levels.



Reduced TEWL after 4 weeks by 33% compared to baseline readings **(Tested at 2.0%)**

Barrier Function.

Enhances moisture retention

References:
1. M, Greener. et al. 2004. Now You're Signaling, With Gas. The Scientist. 26: 105-131
2. D, Liu. et al. 2014. Sulfur dioxide inhibits vascular smooth muscle cell proliferation via suppressing the ERK/MAP kinase pathway mediated by cAMP/PKA signaling. Cell Death and Disease. 5 (1251).
3. Stolz, Patrick, and Barbara Obermayer. "Manufacturing microalgae for skin care." Cosmetics and toiletries 120.3 (2005): 99-106
4. M, Nughes. et al. 2009. Making and working hydrogen sulfide, The chemistry and generation of hydrogen sulfide in vitro and its measurement in vivo: a review. Free Radical Biology & Medicine.1346-1353.

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