

Confidential

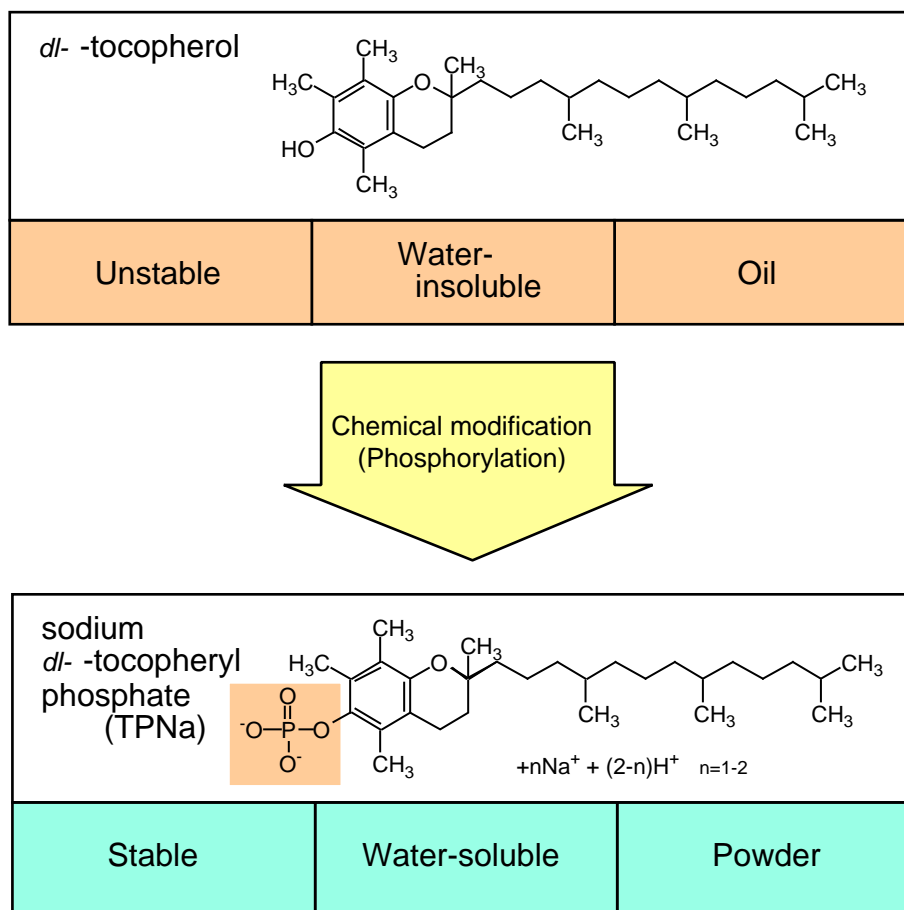
Technical Information  
on  
Vitamin E Phosphate  
sodium *dl* tocopheryl phosphate, TPNa



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&  
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## Vitamin E Modification



### TPNa is a stable, water-soluble powdery vitamin E.

-Tocopherol (vitamin E) is one of the most important vitamins for cosmetics. Acting in harmony with ascorbate (vitamin C) vitamin E protects skin cells from oxidative attacks of radicals and peroxides.

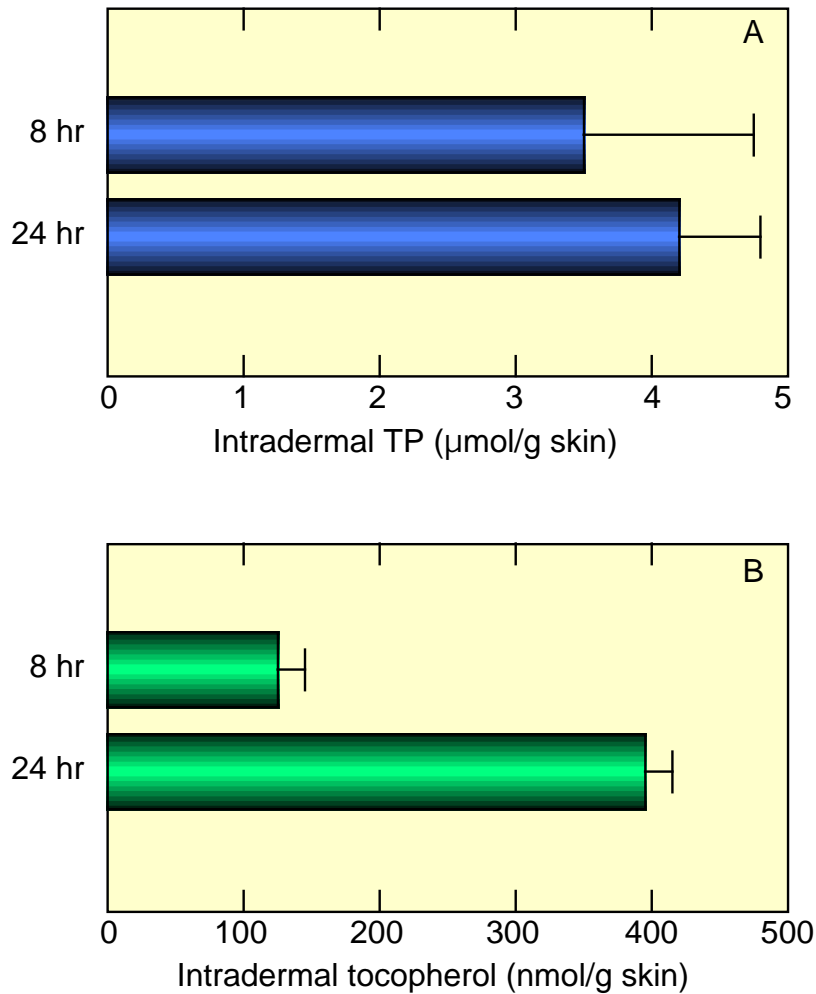
As tocopherol is easily oxidized and quickly loses the reductive activity, tocopheryl acetate, a stable derivative of tocopherol, is popularly formulated. However, its use for aqueous formulations is restricted due to its oily form and poor solubility in water.

Our new product, Vitamin E Phosphate (sodium  $d\text{-}$  -tocopheryl phosphate, TPNa) is a water-soluble powdery vitamin E. The oxygen-sensitive hydroxyl group of tocopherol is chemically modified and protected with a phosphoryl group. Once absorbed in skin, TPNa is readily converted to the active tocopherol via hydrolysis catalyzed by phosphatase, a ubiquitous enzyme in skin.

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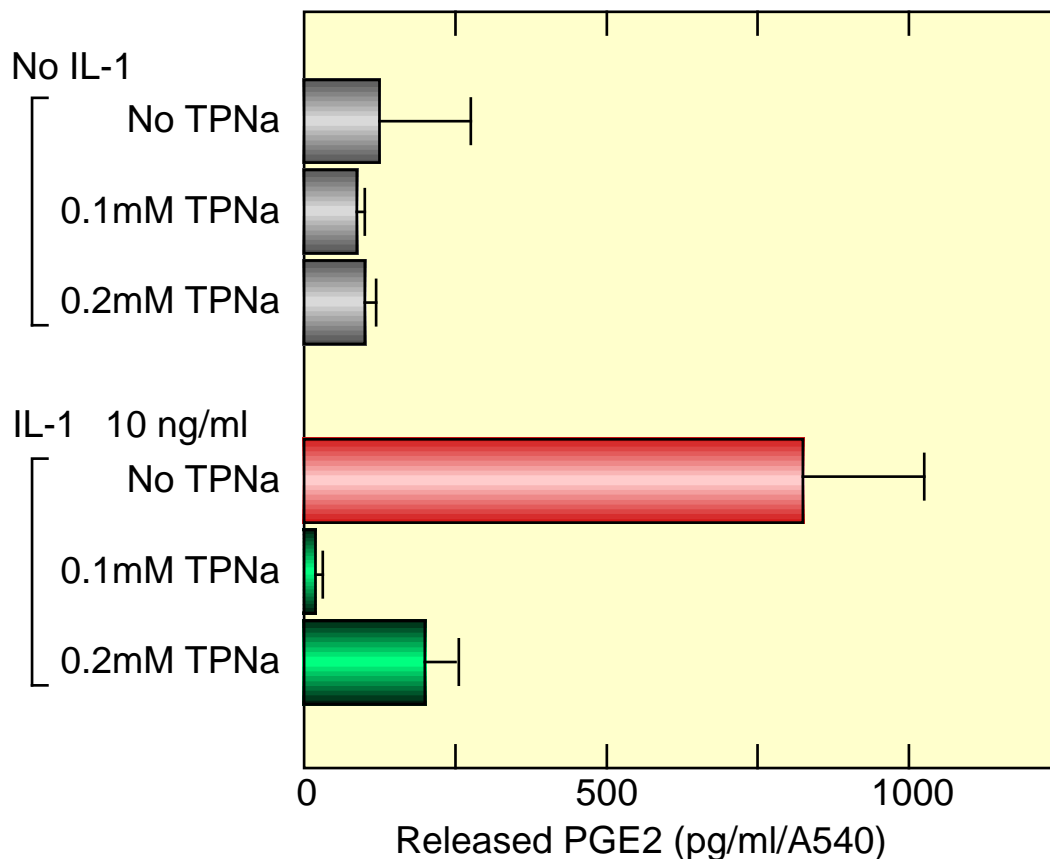
## Permeation and Conversion in Skin Model



The permeation of sodium  $\alpha$ -tocopheryl phosphate (TPNa) and its conversion to tocopherol (Toc) was examined using a three dimensional restructured human skin model (TESTSKIN™ LSE-high, TOYOBO, Japan). TPNa was dissolved in HEPES buffer (pH 7.2) containing 5% ethanol, and 100 $\mu$ l of the solution was applied onto the skin model's surface. After the incubation at 37°C for 8 hours or 24 hours the skin model was homogenized and intradermal concentrations of permeated  $\alpha$ -tocopheryl phosphate (TP) and released Toc were determined by HPLC. As shown above, a quick permeation (A) of applied TPNa and a moderate release (B) of Toc was observed. At 24 hours of incubation about one tenth of permeated TPNa was converted to Toc, which suggested a long-lasting supply of vitamin E in the skin.

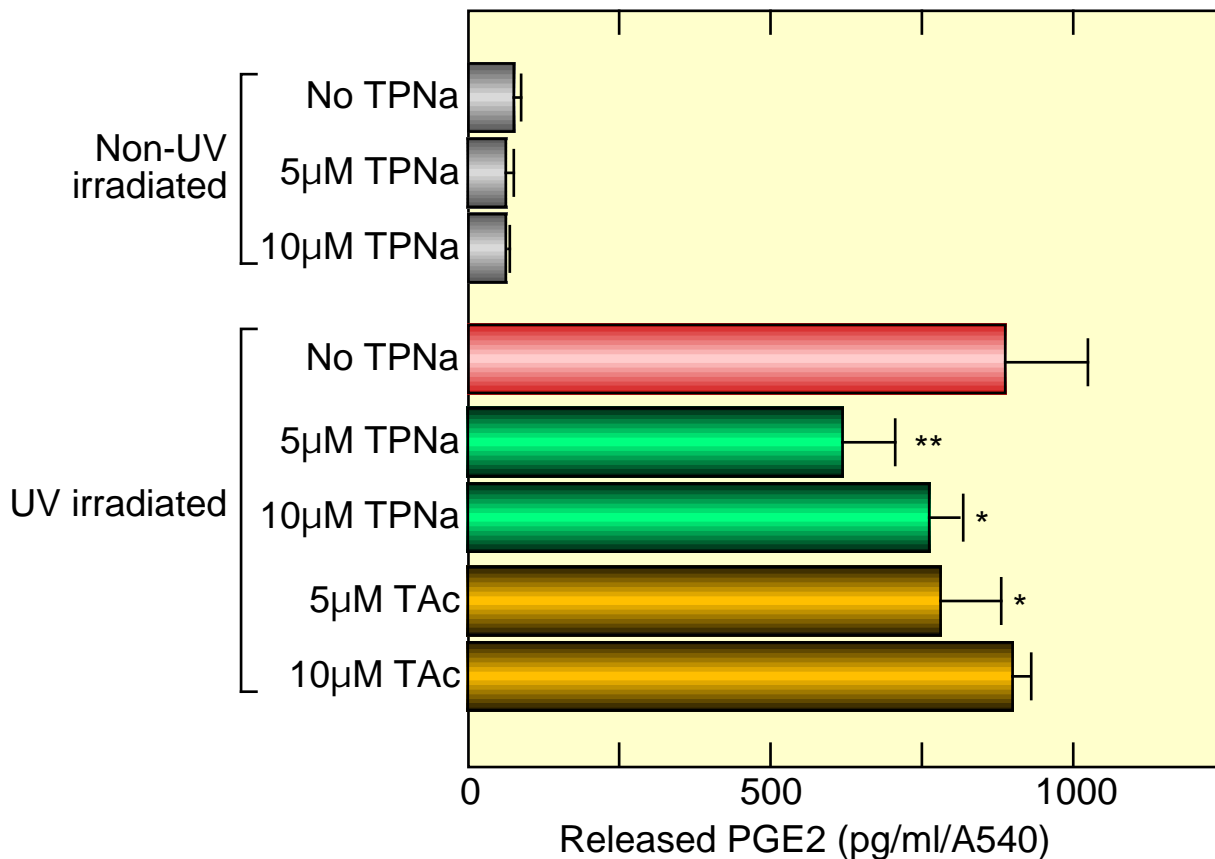


## Prevention of Inflammation



### TPNa suppresses prostaglandin E2 generation.

The suppressive effects of TPNa on inflammation was examined using cell cultivation system. Human keratinocyte SVHK was cultivated until confluent and pretreated with 0.1 mM or 0.2 mM TPNa for twenty four (24) hours. The cells were stimulated by addition of 10 ng/ml of recombinant human interleukin 1-beta (IL-1 ) to trigger the simulated chain reaction of inflammation. After the cultivation in this medium for twenty four hours, the medium was sampled for the measurement of released prostaglandin E2 (PGE2), an inflammation marker, by the ELISA method. The cell amount was spectrometrically determined as absorbance at the wavelength of 570 nm (A570) by Alamar Blue method after the medium sampling. The PGE2 generation was significantly enhanced by addition of IL-1 , while it was suppressed almost completely by the pretreatment with TPNa.



### Suppression of inflammation caused by UV irradiation

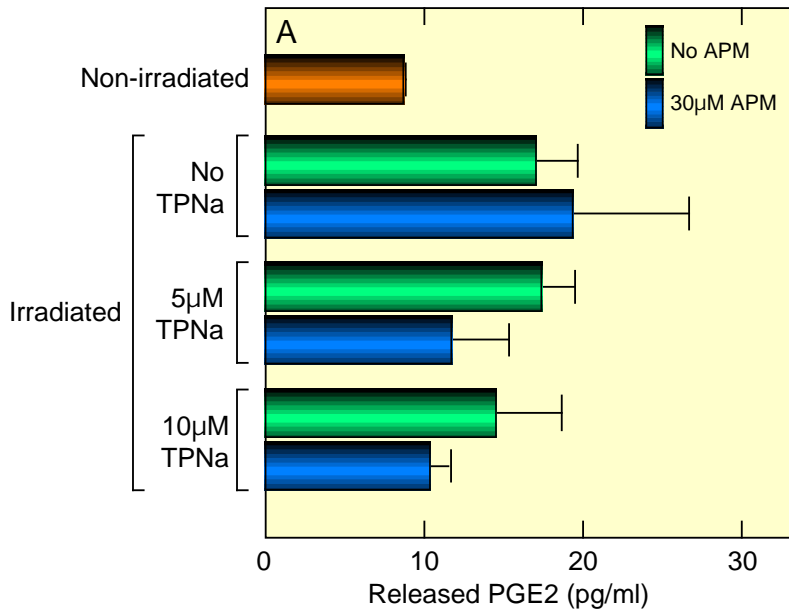
Sodium *dl*-α-tocopheryl phosphate (TPNa) suppresses the inflammation caused by strong UV-B irradiation.

0 to 10 µM TPNa was added to the medium of human keratinocyte grown confluent, and the cells were cultivated for 24 hours. After the medium was replaced with the one containing no TPNa, the cells were irradiated by UVB at an energy of 60 mJ/cm<sup>2</sup>. The amount of secreted prostaglandin E2 (PGE2) was determined by ELISA method after another 24 hours post-cultivation.

Unlike the effects against the IL- induction, very low concentrations of TPNa pretreatment gave the significant suppression of inflammation. The weaker suppression was observed by the pretreatment with tocopheryl acetate (TAc) at the same concentration.

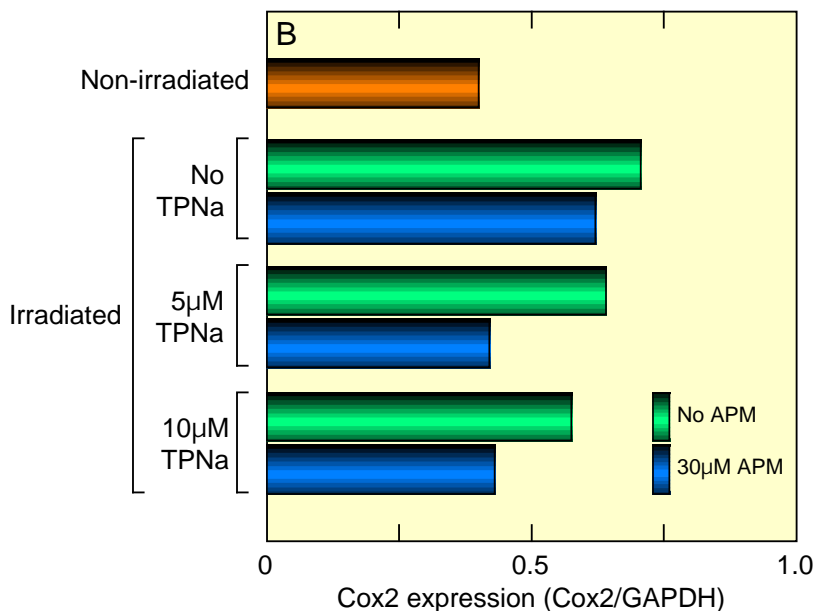


## Synergy with Vitamin C



The synergistic effects of sodium vitamin E phosphate (sodium  $\alpha$ -tocopheryl phosphate, TPNa) with vitamin C phosphate (Magnesium ascorbyl phosphate, APM) on inflammation was examined in a cell cultivation system.

Human keratinocyte (SVHK) was cultivated in the medium containing 0, 5, or 10  $\mu$ M TPNa for 24 hours. After the cultivation the medium was replaced with phosphate buffer, and the cells were irradiated by UVB (30 mJ/cm<sup>2</sup>). The concentration of released prostaglandin E2 (PGE2) in the buffer was determined by ELISA method after another 24 hour-incubation with or without 30  $\mu$ M APM.



Supplementation of APM significantly enhanced the suppressive effects of TPNa on PGE2 generation. The PGE2 level of the cells treated with 10  $\mu$ M TPNa and 30  $\mu$ M APM remained almost the same as that of non-UVB irradiated cells (A).

No suppressive effect was observed in APM-alone treated cells, which suggested that vitamin C reacted on vitamin E but not on the irradiation cascade directly.

The expression of cyclooxygenase (Cox2) gene, which was responsible for the inflammation, was also examined in the same experimental system. The expression level showed a good correlation with PGE2 concentration in each tested condition (B).