New Generation
Vitamin C Derivative
CORUM 9515
3-O-Ethyl Ascorbic Acid

AS POTENT AS VITAMIN C WITH BETTER STABILITY
CORUM 9515
INCI: 3- O – Ethyl ascorbic acid

Also known as:
- 3- O – ethyl ascorbic ether
- 3- O – ethyl ascorbic acid

Physical Properties

- Purity > 99.0%
- White crystalline powder
- Water soluble
- Metabolized by the human body in the same manner as L- ascorbic acid (Vitamin C)
- Excellent stability

CAS: 86404-04-8
MW: 204.18
The Functions of **CORUM 9515**

- Skin lightening & Balance skin tone
- Reduce dark spot & age spot
- Anti-photoaging
- Collagen synthesis
- Reversing auto-oxidation
- Radical scavenging
- DNA protection
- Easy to penetrate the epidermis
CORUM 9515 Efficacy Studies

- In-vitro tyrosinase inhibition
- Reducing activity
- In-vitro whitening activity
- Ex-vivo melanin assay
- In-vivo whitening efficacy

- Anti-photoaging (Anti-inflammation test)
- Stimulation of collagen synthesis
- Radical scavenging effect
- DNA protection
- Skin penetration profile
CORUM 9515 Whitening Mechanism

Three melanin-generating enzymes:
1. Tyrosinase
2. TRP-2 (DOPAchrome tautomerase)
3. TRP-1 (DHICA oxidase)

- Inhibit the reaction center Cu$^{+2}$ ion of tyrosinase
- Inhibit the activity of TRP-2
- Reduce oxidized melanin pigments

Efficacy Test: *In-vitro* tyrosinase (I) inhibition

**Reaction:**

Tyrosine Assay

**Tyrosine Inhibition Activity IC50**

0.1 % CORUM 9515 can inhibit tyrosinase activity up to 37.6 %.

2 mg/mL of CORUM 9515 can reach 50% inhibition.

Efficacy Test: *In-vitro* tyrosinase (II) inhibition

**Reaction:**

- **DOPA**
  - HO
  - HO
  - CH₂
  - NH₂
  - COOH

- **Dopaquinone**
  - O
  - C₆H₄
  - CH₂
  - NH₂
  - COOH

**DOPA Assay**

- **Vit.C:** 99.1%
- **Corum 9515:** 54.8%
- **AA2G:** 22.3%
- **MAP:** 31.9%
- **α-Arbutin:** -19.9%

Sample: 1 mg/ml (0.1%)

**Tyrosine Inhibition Activity IC50**

- 2 mg/ml: 67.1%
- 4 mg/ml: 83.3%
- 8 mg/ml: 92.9%

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mg/ml</td>
<td>40.7%</td>
</tr>
<tr>
<td>1 mg/ml</td>
<td>47.1%</td>
</tr>
<tr>
<td>2 mg/ml</td>
<td>67.1%</td>
</tr>
<tr>
<td>4 mg/ml</td>
<td>83.3%</td>
</tr>
<tr>
<td>8 mg/ml</td>
<td>92.9%</td>
</tr>
</tbody>
</table>

**Ref:** Corum Internal Report. Feb 2009.

0.1 % Corum 9515 can inhibit tyrosinase activity up to 54.8%.

1 mg/mL of Corum 9515 can reach 50% inhibition.
Efficacy Test: *In-vitro Tyrosinase*, Trp-2 inhibition

**Tyrosinase inhibition**

Result: 1% Corum 9515 can inhibit tyrosinase activity up to 47.5% in protein level.

Efficacy Test: *In-vitro* Tyrosinase, **Trp-2** inhibition

Result: 1% Corum 9515 can inhibit Trp-2 activity up to 72.6% in protein level.

CORUM 9515 Efficacy Studies

- **In-vitro tyrosinase inhibition**
- **Reducing activity**
- **In-vitro whitening activity**
- **Ex-vivo melanin assay**
- **In-vivo whitening efficacy**

- **Anti-photoaging**
  (Anti-inflammation test)
- **Stimulation of collagen synthesis**
- **Radical scavenging effect**
- **DNA protection**
- **Skin penetration profile**

CORUM
Advance through Knowledge
Efficacy Test: Ferric Reducing Ability

Efficacy Test: Ferric Reducing Ability

- pKa value greater - give electron easier - better reducing ability

- 2’ carbon without ethyl group bonded has better reducing ability

Ascorbic acid (reduced form of Vitamin C)

Ethyl Ascorbic Acid
MW: 204.18

AA2G
MW: 338.26

MAP
MW: 759.22
Efficacy Test: Ferric Reducing Ability

\[
\text{Fe}^{3+} + e^- \rightarrow \text{Fe}^{2+}
\]

Sample: 10 mg/ml (1%)

CORUM 9515 Efficacy Studies

- **In-vitro** tyrosinase inhibition
- Reducing activity
- **In-vitro** whitening activity
- Ex-vivo melanin assay
- **In-vivo** whitening efficacy

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  (Anti-inflammation test)
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- DNA protection
- Skin penetration profile
Efficacy Test:

*In-vitro* whitening activity study - melanin assessment by IDEA

Method: 12 hours contact with the product and stimulation during 48 hours with theophylline at 0.5 mM.

Result: 49.75% whitening effect, 20mg/ml Corum 9515

CORUM 9515 Efficacy Studies

- In-vitro tyrosinase inhibition
- Reducing activity
- In-vitro whitening activity
- Ex-vivo melanin assay
- In-vivo whitening efficacy

- Anti-photoaging (Anti-inflammation test)
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- Skin penetration profile
Efficacy Test: Ex-vivo Melanin Assay

**MelanoDerm™**

by BioInnovation Laboratories, Inc (USA)

**Purpose**
- using an *in-vitro* tissue model of the human epidermis prepared from cultured human keratinocytes and melanocytes.
- both water-soluble and water-insoluble materials
- skin darkening agents or skin lightening agents
Efficacy Test: Ex-vivo Melanin Assay

Untreated Tissue

Day 0

Day 9

3 % Corum 9515

Melanin Content (μg/tissue)

<table>
<thead>
<tr>
<th>Day</th>
<th>Melanin (μg/tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>23.4</td>
</tr>
<tr>
<td>3 % Corum 9515</td>
<td>16.7</td>
</tr>
</tbody>
</table>
CORUM 9515 Efficacy Studies

- In-vitro tyrosinase inhibition
- Reducing activity
- In-vitro whitening activity
- Ex-vivo melanin assay
- In-vivo whitening efficacy
- Anti-photoaging (Anti-inflammation test)
- Stimulation of collagen synthesis
- Radical scavenging effect
- DNA protection
- Skin penetration profile
Efficacy Test: *In-vivo* whitening efficacy
by *SPINCONTROL*

Method: Chromametric Analysis

Efficacy Test: *In-vivo* whitening efficacy by *SPINCONTROL*

**Volunteers:**
- 20 healthy Asian
- Female
- 25-40 years old
- Skin type III

**Method:**
- Chromametry (CR-300)
- 2% Corum 9515 cream

**Result:** Significant increase on mean ITA

Efficacy Test: *In-vivo* whitening efficacy

Efficacy Test: *In-vivo* whitening efficacy

CORUM 9515 Efficacy Studies

- **In-vitro tyrosinase inhibition**
- Reducing activity
- **In-vitro whitening activity**
- **Ex-vivo melanin assay**
- **In-vivo whitening efficacy**

- Anti-photoaging (Anti-inflammation test)
- Stimulation of collagen synthesis
- Radical scavenging effect
- DNA protection
- Skin penetration profile
Mechanism of Photoaging

- UVA, UVB
- Epidermis
- ROS
- IL-6
- MMP-1
- Stratum Corneum
- Dermatocytes
- Blood Vessels
- Collagen degradation
- Wrinkle formation

Dermis
- Elastin
- Collagen

Subcutis
Efficacy Test: Anti-inflammation IL-6 test

Principle

Stimulate by External Material (e.g. 40 mJ/cm² UVB)

Cytokines Release

Regulate Inflammatory reaction

Corum 9515

IL-6 Production (%)

UVB irradiated

Control 1

Control 2

1% Corum 9515

0.5% Corum 9515

0.25% Corum 9515

100% Inhibition rate

CORUM 9515 Efficacy Studies

- *In-vitro* tyrosinase inhibition
- Reducing activity
- *In-vitro* whitening activity
- *Ex-vivo* melanin assay
- *In-vivo* whitening efficacy

- Anti-photoaging
  (Anti-inflammation test)
- Stimulation of collagen synthesis
- Radical scavenging effect
- DNA protection
- Skin penetration profile
Collagen and its function

**What is collagen**
- The main protein of connective tissue
- Make up 25% - 35% of the whole-body protein content
- Different types of collagen

**Functions**
- Impart strength, support and skin elasticity

**Why important**
- As skin ages, it produces less collagen and loses its elasticity
  
  Wrinkle formation + Aged skin

**Diagram:**
- Abundant collagen in Epidermis and Dermis
- Less collagen in Epidermis and Dermis
The role of ascorbic acid in collagen synthesis

- Proline residues convert into hydroxyproline during the synthesis of collagen.
- Hydroxylation requires Vitamin C.
Efficacy Test:
Stimulation of natural collagen synthesis

by IDEA, France
After 48hrs contacts

- TGF β1 is a very strong collagen synthesis stimulator
- It is tested with type I collagen

Result: Corum 9515 has a similar effect on collagen synthesis as TGF β1

CORUM 9515 Efficacy Studies

- In-vitro tyrosinase inhibition
- Reducing activity
- In-vitro whitening activity
- Ex-vivo melanin assay
- In-vivo whitening efficacy

- Anti-photoaging
  (Anti-inflammation test)
- Stimulation of collagen synthesis
- Radical scavenging effect
- DNA protection
- Skin penetration profile
Corum 9515 Free-Radical Scavenging Mechanism

**What are free radicals**
- Atoms, molecules, or ions with unpaired electrons
- Due to its unpaired electrons, they are often highly reactive
- Often causes chain reactions

**Cause**
- UV rays, stress & environmental pollution etc.

**Why important**
- It can participate in unwanted side reactions resulting in cell damage
- Many form of cancer are thought to be the result of reactions between free radicals and DNA

Efficacy Test: Radical scavenging test
ROS assay after UVB irradiated

**Principle**

CORUM 9515 shows great ability on ROS inhibition.

CORUM 9515 Efficacy Studies

- *In-vitro* tyrosinase inhibition
- Reducing activity
- *In-vitro* whitening activity
- *Ex-vivo* melanin assay
- *In-vivo* whitening efficacy
- Anti-photoaging (Anti-inflammation test)
- Stimulation of collagen synthesis
- Radical scavenging effect
- DNA protection
  a. *In-vitro*, Comet Assay
  b. *Ex-vivo*
- Skin penetration profile
Efficacy Test: DNA Protection

Comet Assay

- **Method:** Single Cell Gel Electrophoresis Assay – Comet Assay
- **Theory:**

  ![Diagram of DNA damage and electrophoresis](image)

  - **Strong alkali**
  - **DNA damaged by H$_2$O$_2$**
  - **DNA disintegrates**

  - Single cell electrophoresis

  ![Images showing DNA damage](image)

  - **No damage**
  - **Serious damage**
Efficacy Test: DNA Protection

Comet Assay

Result: CORUM 9515 shows high efficiency on DNA protection activity.
What are Sun Burn Cells?

Sunburn cells (SBC) are keratinocytes undergoing apoptosis as a protective mechanism against the carcinogenic effects of UVB irradiation which irreversibly damages their DNA or other chromophores.

Sun burn cells are indicated by arrow.
**Efficacy Test: DNA Protection after UVB irradiation on human living skin explants**

by BIO-EC, France

**Explants preparation:**
- From a 45-year-old Caucasian woman
- 12 skin explants of an average diameter of 11 mm (±1mm)

**Number of sun burn cell in the epidermis**

<table>
<thead>
<tr>
<th></th>
<th>Number of Sunburn Cells/cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>53.3</td>
</tr>
<tr>
<td>4% Corum 9515 with UV irradiation</td>
<td>35.4</td>
</tr>
</tbody>
</table>

Result: Corum 9515 shows high efficiency on DNA protection activity after UVB irradiation.
CORUM 9515 Efficacy Studies

- **In-vitro** tyrosinase inhibition
- Reducing activity
- **In-vitro** whitening activity
- **Ex-vivo** melanin assay
- **In-vivo** whitening efficacy
- Anti-photoaging
  (Anti-inflammation test)
- Stimulation of collagen synthesis
- Radical scavenging effect
- DNA protection
- Skin penetration profile
Skin Penetration Profile

Human Epidermis Model EPISKIN®: In Vitro Skin Penetration Test

*In vitro* reconstructed human epidermis which is similar to the *in vivo* human epidermis.

※ Episkin is a skin model as alternatives to animal testing.

Applications:

- Percutaneous absorption
- *In vitro* skin corrosion
- *In vitro* skin irritation
- Pharmacology /Toxicology

Test materials:

- 2 % solution
- 2 % Whitening cream
Skin Penetration Profile

Human Epidermis Model EPISKIN® : In Vitro Skin Penetration Test
Cumulated permeated amount

Test materials: 2% solution

Result: CORUM 9515 shows better skin penetration ability compared with AA2G.
Skin Penetration Profile

Human Epidermis Model EPISKIN®: In Vitro Skin Penetration Test

Penetration rate • Ratio (Corum 9515 vs. AA2G)

Test materials: 2% solution

Result: CORUM 9515 shows better skin penetration ability compared with AA2G.
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Skin Penetration Profile

Human Epidermis Model EPISKIN® : In Vitro Skin Penetration Test

Penetration rate • Ratio (Corum 9515 vs. AA2G)

Test materials: 2 % Cream

Result: CORUM 9515 shows better skin penetration ability compared with AA2G.
Stability Tests

- **Heat-stability (45°C, 1 month)**
  - color (Transmittance in 440nm)
  - purity (HPLC)

- **Photo-stability (sunlight, 1 month)**

- **Buffer system and pH effect (45°C, 56 days and RT, 90 days)**
Stability: Heat-Stability

0 month 2% 45°C without buffer

1 month 2% 45°C without buffer

1 Month 45°C Stability
(Transmittance at 440nm)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Transmittance %T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corum 9515</td>
<td>96.8</td>
</tr>
<tr>
<td>α-Arbutin</td>
<td>90.9</td>
</tr>
<tr>
<td>AA2G</td>
<td>85.1</td>
</tr>
<tr>
<td>VitC</td>
<td>41.6</td>
</tr>
</tbody>
</table>

Stability: Crystalline Powder Heat-Stability

60 Days 45°C Purity

- HPLC system
- UV detection at 214nm
- Corum 9515 crystalline powder

Result: CORUM 9515 crystalline powder remains stable under 45°C for 60 days

Stability Tests

- **Heat-stability (45°C, 1 month)**
  - color (Transmittance in 440nm)
  - purity (HPLC)

- **Photo-stability (sunlight, 1 month)**

- **Buffer system and pH effect (45°C, 56 days and RT, 90 days)**
Stability: CORUM 9515 Photo-Stability

0 month 2% under sunlight without buffer

1 month 2% under sunlight without buffer

Stability Tests

- **Heat-stability** (45°C, 1 month)
  - color (Transmittance in 440nm)
  - purity (HPLC)

- **Photo-stability** (sunlight, 1 month)

- **Buffer system and pH effect** (45°C, 56 days and RT, 90 days)
Stability under different pH & buffer

**PURITY** assay by HPLC

**Preparation:**
2% buffer solution

**Day:** 56 days at 45°C

**Buffer system:**
A - Sodium Citrate – Citric Acid
B - Na2HPO4- Citric Acid
C - NaHCO3 – Citric Acid

pH Stability under different buffer

pH Stability

Preparation:
2% buffer solution

Day: 56 days at 45°C

Buffer system:
A - Sodium Citrate - Citric Acid
B - Na2HPO4 - Citric Acid
C - NaHCO3 - Citric Acid
D - NaOH - Citric acid

Corum 9515 Ethyl Ascorbic Acid is a powerful anti-oxidant

Thus a **stronger Anti-oxidant** is recommended to be used in formulation → prevent losing Corum 9515’s effect before it reaches its target area.
Anti-oxidant lessen color change

45°C for 6 months

<table>
<thead>
<tr>
<th>C-9515</th>
<th>Control</th>
<th>2%</th>
<th>2%</th>
<th>2%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium bisulfite</td>
<td>0%</td>
<td>0%</td>
<td>0.15%</td>
<td>0.2%</td>
</tr>
</tbody>
</table>

- Sodium bisulfite can lessen color change.

- But ! **Too much** sodium bisulfite will **accelerate** color change.
Minimize Color Change - Chelating Agent

**Metal Ions** promotes oxidation. Chelating agents work to trap and inactivate these metal ions with the formation of chelating complexes in the solution.

**EDTMP** was found to minimize oxidation remarkably in solutions containing Corum 9515 according to our experiments.

![Graph showing transmittance over days](image)
CORUM 9515 Toxicological Information

- **Skin Irritancy Test by IDEA, France**

  2% CORUM 9515 on the external face of the arm maintained over 48 hours with the help of a semi-occlusive patch.

  CORUM 9515 is found to be **non-irritant** after 48 hours semi-occlusive patch test.

- **Cytotoxicity Test by Evic, France**

  Cytotoxicity test on CORUM 9515 diluted with 10% distilled water.

  CORUM 9515 diluted at 10% with distilled water was judged **negligible**.

- **AMES Test by Vivotecnia, Spain**

  CORUM 9515 were found to be **non mutagenic** and **non pro-mutagenic**.
CORUM 9515 Summary

- **Effective** and **stable** skin lightening agent
- Balance the skin tone
- Reduce dark spot
- Prevent photoaging
- Increase collagen synthesis
- Excellent anti-oxidation properties
- Scavenge radical
- DNA protection
Thank you for your attention!