麦角硫因
延 缓 衰 老

Youthful Skin - Ergothioneine

Targeting intelligently mitochondria and nucleus, Long-acting, and Natural Antioxidant

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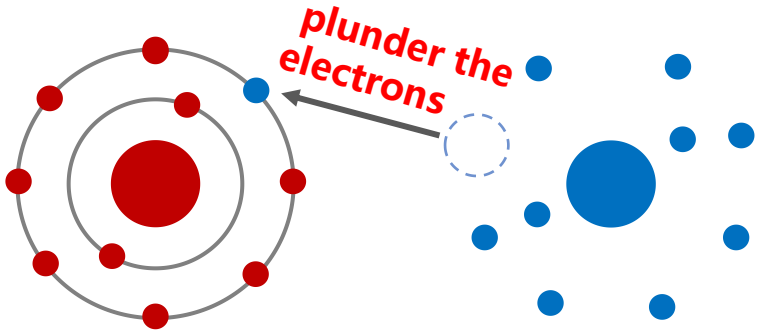
01

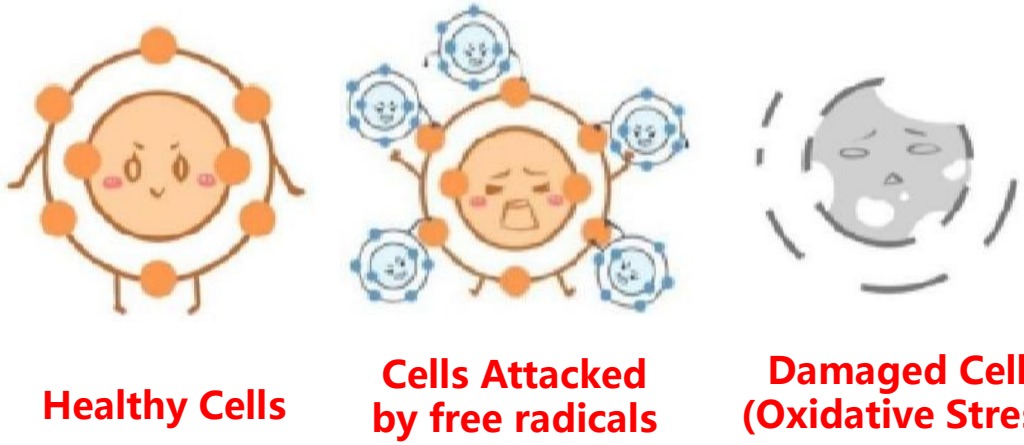
Mechanism of Oxidation

- Oxidation signals of skin : oily and dry, lack of elasticity, slow recovery after staying up late, obvious fine lines, difficult to absorb nutrients, larger pores, deeper sunspots, tissue collapse, facial sagging, and obvious lip corner lines and nasolabial folds.

Definition of Free Radicals

Free radicals are groups in cells with unpaired electrons that "plunder" the electrons of normal molecules or atoms, and destroy normal cells. Free radicals are highly active and react with almost everything in the organism and produce more free radicals. Life activities are inseparable from free radicals, but excessive free radicals are the culprits of aging and disease. The damage of free radicals to the human body mainly has three aspects: damage to cell membranes; Inactivation of serum antiproteases; Cause cell mutation and accumulation of damaged genes.

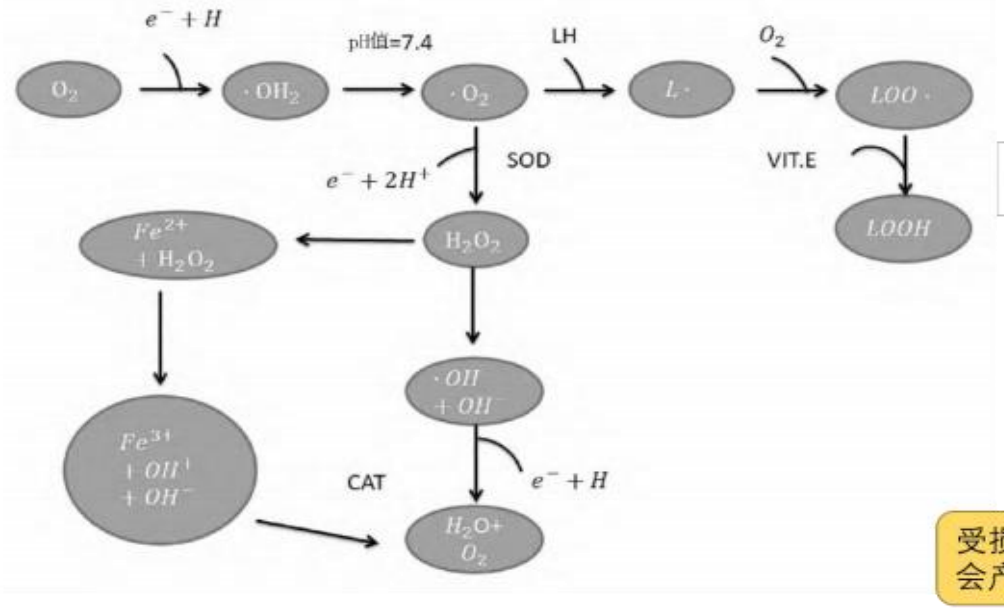


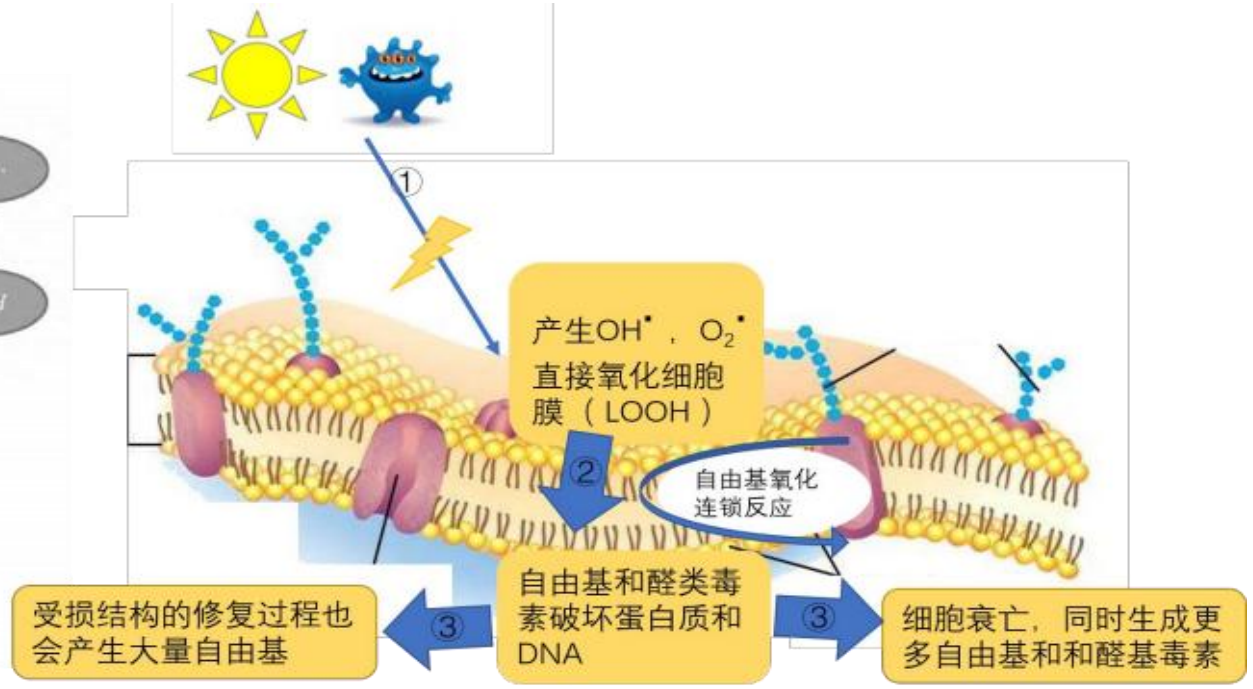


Antioxidant is the basis for whitening, lightening spots, anti-aging

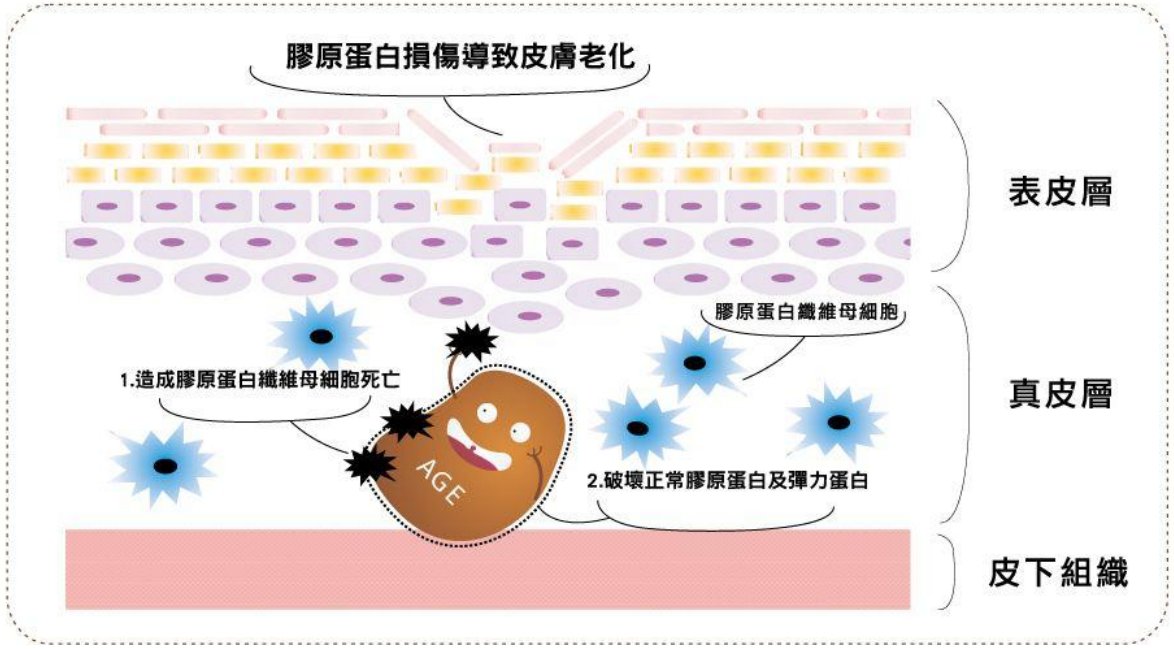
(Oxidative Stress)

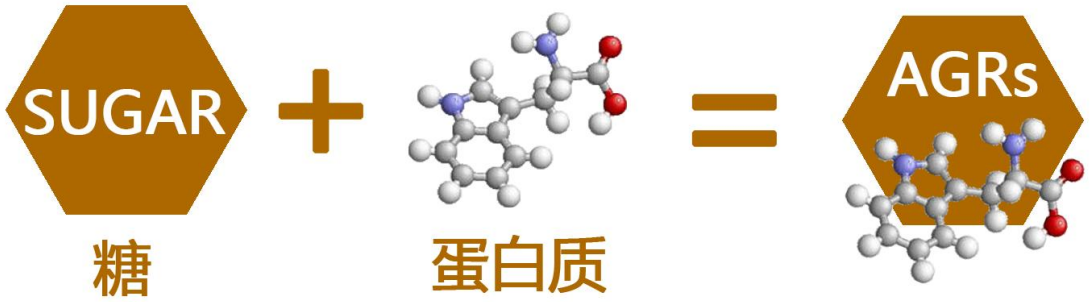
- Under normal conditions, the production and elimination of free radicals in the body are in a dynamic balance. Free-radical defense enzymes in the body, such as superoxide dismutase (SOD) and catalase (CAT), could remove excess free radicals. With age or poor lifestyle habits, this balance can be more easily disrupted, and will lead to the production of excess free radicals.
- Free radicals are highly destructive. After a series of oxidation reactions, it usually leads to changes in cell structure and function.





Mechanism of Oxidation--Saccharification Reaction

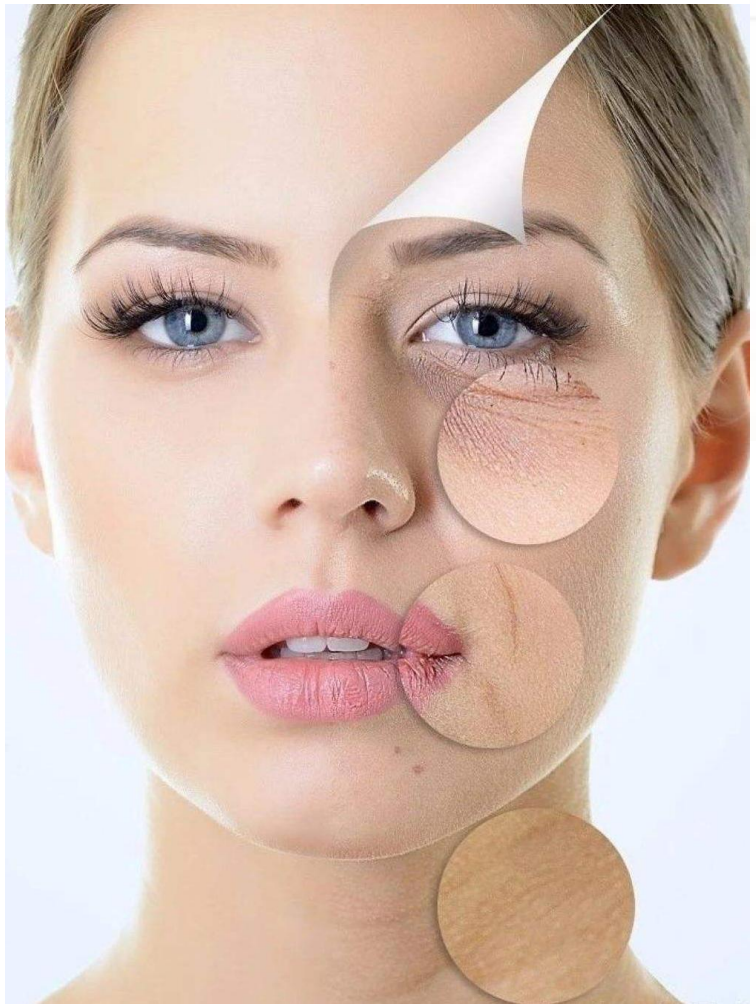




Saccharification Reaction

- Saccharification reaction is the process by which sugar molecules are combined with proteins or fat molecules to form AGRs when there is no enzymes and no additional energy.
- It also induces inflammation and keeps immune cells working, and most commonly caused pimples on our faces. The Saccharification reaction will induce the production of a large number of free radicals, increase the burden on the body's antioxidant system, and also stimulate the production of melanin, making the skin yellow and dark.

Mechanism of Oxidation--Antioxidant = Anti free radical



Antioxidantion = Anti Free Radicals

Too many free radicals are the culprit of aging and disease. Free radicals are mainly produced in the mitochondria of cells. To fight against the free radicals, it is necessary to find long-acting antioxidants that can enter into the mitochondria and remove permanently from the source the excessive free radicals produced by the mitochondria.

Mechanism of Oxidation--Antioxidant = Anti free radical

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- Glutathione (GSH)
- Idebenone
- Superoxide dismutase (SOD)
- Coenzyme Q10
- Vitamin A/C/E
- Polyphenols (resveratrol)
- Ferulic acid (3-methoxy-4-hydroxycinnamic acid)
- Lipoic acid
- Astaxanthin
- Fullerene (C60)

It has been demonstrated in many articles that Ergothioneine, as a natural antioxidant, could protect DNA and proteins from oxidation. In many biochemical reactions at cellular level, ergothioneine is thought to be 6,000 times more potent than vitamin E.

The latest generation of natural and powerful antioxidant — Ergothioneine

Antioxidant family

The firsts generation

Vitamins A, C, E

01

The second generation

β-Carotene, SOD, Coenzyme Q10 (derivatized and modified to Idebenone)

02

The third generation

Glutathione GSH, Ferulic Acid, Flavonoids (Rutin), Polyphenols (Resveratrol), Anthocyanins (OPC), Grape Seed, Blueberry Extract, Green Tea, Alpha Lipoic Acid, Lycopene, Astaxanthin

03

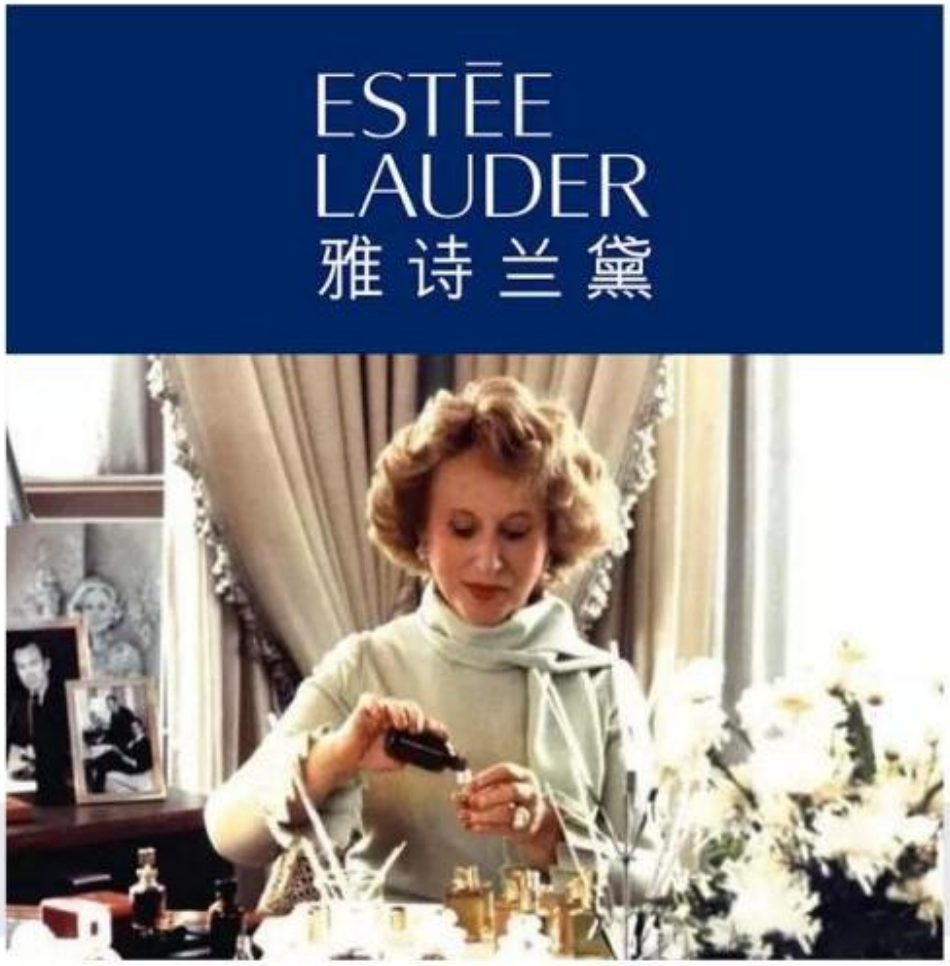
The fourth generation

Glabridin, ectoine, carnosine, EUK134, fullerenes

04

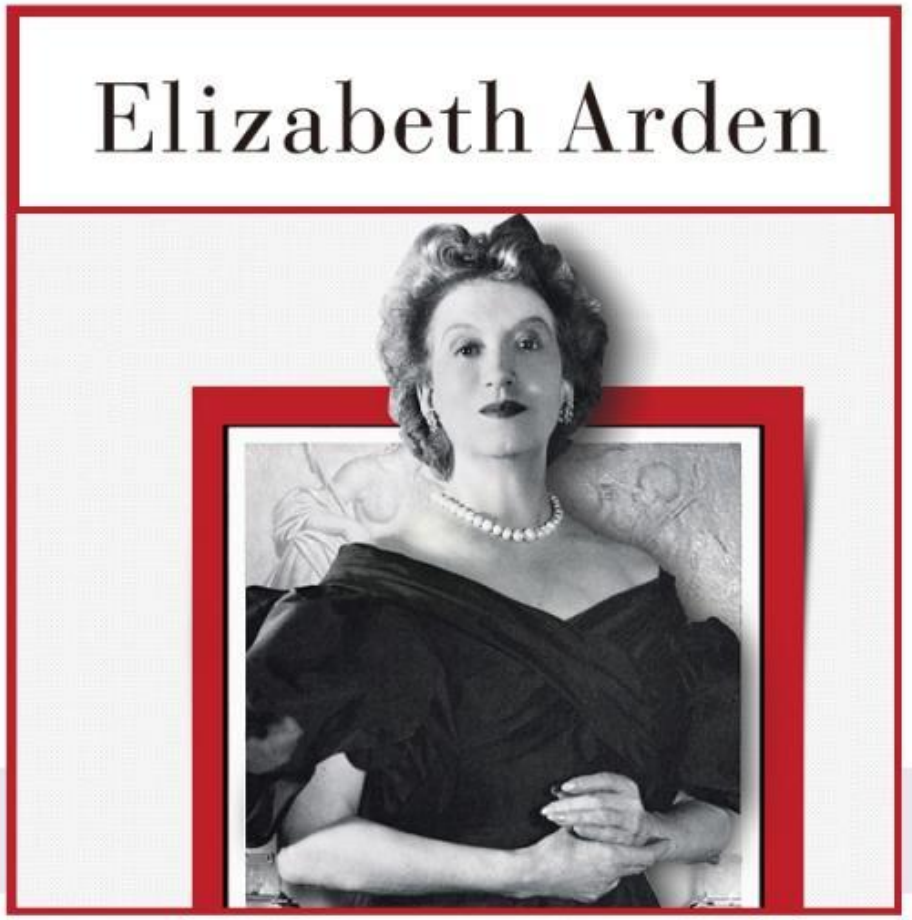
Mechanism of Oxidation -- Market Application

Estee Lauder has added EGT to various products such as Clinique, Origins and LA MER.



Mechanism of Oxidation--Market Application

Elizabeth Arden's PREVAGE® Anti-aging Daily Serum also contains EGT as one of the most important ingredients.



Mechanism of Oxidation--Market Application

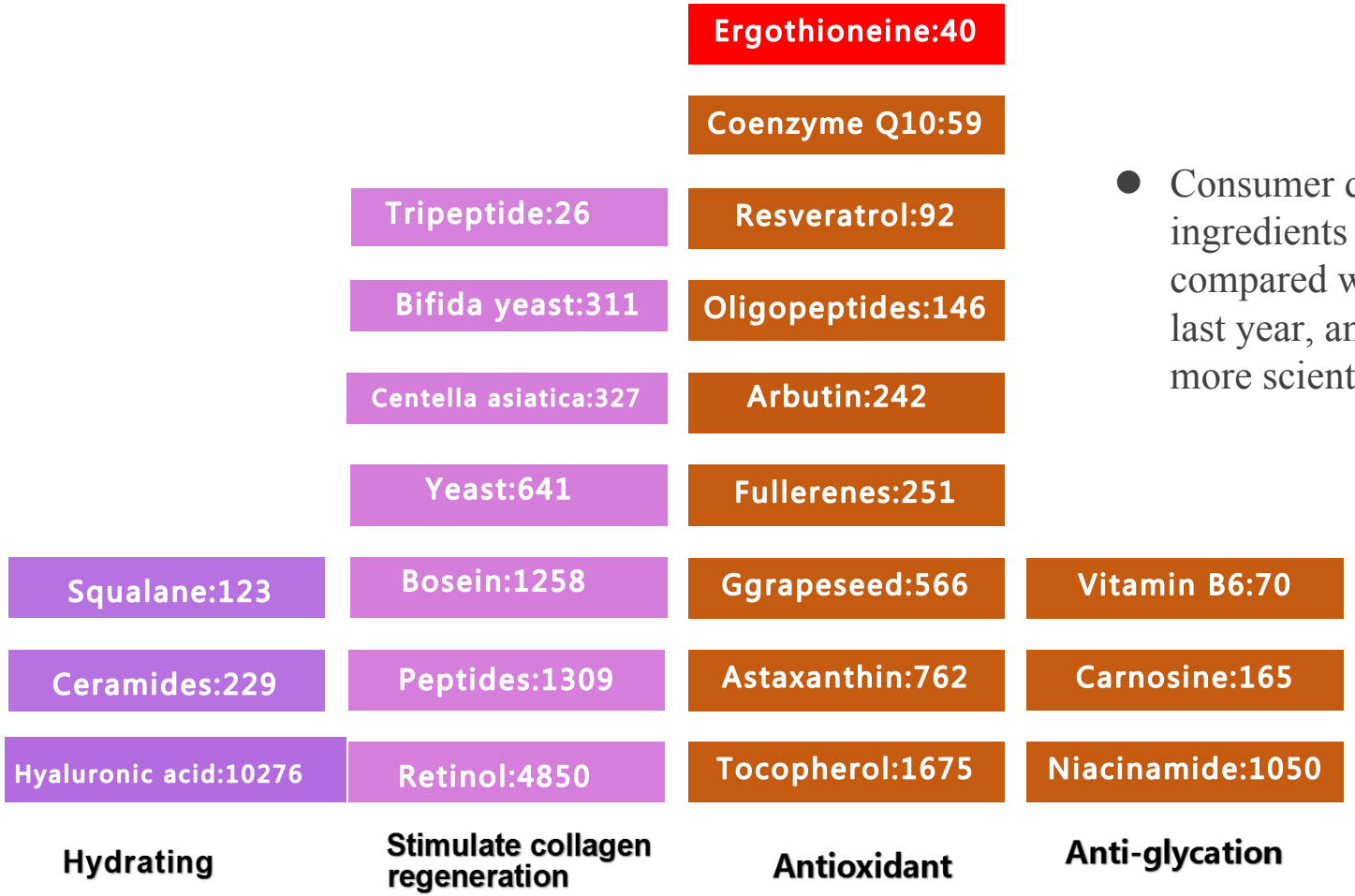
NEOVA Smart Skincare regard EGT as a safe biological sunscreen, and has added EGT to its full range of products .

Mechanism of Oxidation--Market Application

ANTI-AGING

In China a new aggressvie brand Guyu, use EGT as antioxidant and anti-inflammatory in Lightening Skin Toner, only 28 days, make you white and lightening

2022 H1 Social Media Consumer Mention Frequency of Anti-aging Ingredient



- Consumer discussion of anti-aging ingredients increased by 91% compared with the same period of last year, and it becomes more and more scientific in anti-aging.

Data source: Jigua Data (www.ji-gua.com), statistical time: 2021.01-2022.06; Statistical platforms: Douyin, Kuaishou; (Note: Sales data is estimated)

Mechanism of Oxidation--Market Application

ANTI-AGING

Ergothione rises rapidly and debuts in the Center of anti-aging world.

TOP	Product name	Anti-aging mechanism	Branding	H1 Sales Index 2022	H1 Sales Index 2021	Year increase
1	Sodium hyaluronate (hyaluronic acid)	Stimulates collagen regeneration	L'Oréal Paris, Runbaiyan, Dr. Ling, etc	625.17	140.29	346%
2	Bosein	Stimulates GAGs synthesis	'Oréal Paris, HBN, Uemi Fuyo, etc	215.78	97.20	122%
3	Retinol (A alcohol)	Stimulates collagen regeneration	Proya, Bloomage Biotech, HBN, etc	79.73	71.68	11%
4	Copper peptides	Stimulates GAGs synthesis	Winona, One Issue, Code of the Beast, etc	68.34	21.33	220%
5	Fullerenes	Scavenges excess free radicals	Delai, Renhe, Neutrogena, etc	54.57	50.54	8%
6	Hexapeptide	Blocks muscle nerve transmission	Proya, Estée Lauder, Ou Shiman, etc	54.96	26.15	110%
7	Astaxanthin	Scavenges excess free radicals	Estée Lauder, Hanshu, DHC, etc	49.06	8.47	479%
8	Astaxanthin	Scavenges excess free radicals	Olay, Shu Li Ke, Ou Shiman, etc	28.73	22.58	27%
9	Bifida yeast	Stimulates collagen regeneration	Estée Lauder, Lancôme, Clinique, etc	21.85	16.79	30%
10	Carnosine	Scavenges excess free radicals	HFP, Dr. Yaoer, Yi Lian, etc	15.23	6.18	146%
11	Ginseng	Scavenges excess free radicals	Sulwhasoo, Mao Geping, Weather Dan, etc	10.14	10.57	-4%
12	Arbutin	Scavenges excess free radicals	HBN, Winona, The Ordinary, etc	8.11	0.90	805%
13	Ceramides	Stimulates collagen regeneration	Arden, Runbaiyan, Yuze, etc	7.98	1.92	315%
14	Tocopherol (Vitamin E)	Scavenges excess free radicals	Renhe, Vaseline, Aojiabao, etc	7.82	1.32	491%
15	Oligopeptides	Blocks muscle nerve transmission	Olay, HFP, Cidan, etc	7.59	2.82	169%
16	Centella asiatica	Scavenges excess free radicals	L'Oréal, La Roche-Posay, Kiehl's, etc	6.22	3.39	83%
17	Resveratrol	Scavenges excess free radicals	Shiseido, The Ordinary, Shuliko and others	5.52	11.49	-52%
18	caffeine	Scavenges excess free radicals	HBN, Lifelong Research, Nature Hall, etc	5.31	0.51	936%
19	**Ergothioneine**	Scavenges excess free radicals	Estée Lauder, Clinique, Proya, etc	3.36	0.02	**20702%**
20	Tripeptide	Blocks muscle nerve transmission	Rantei , Krittina and others	2.95	0.00	158610%

Data source: Jigua Data (www.ji-gua.com), statistical time: 2021.01-2022.06; Statistical platforms: Douyin, Kuaishou; (Note: Sales data is estimated)

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Efficacy of Ergothioneine

02

- Ergothioneine is a rare natural chiral amino acid with strong functions of scavenging free radical , whitening, anti-aging and detoxification.
- Ergothione was firstly isolated from ergot in 1909, that is how its name came. At present, it has been found that it can be synthesized in most fungi, some mushrooms, streptococcus, mycobacteria and other microorganisms and can be absorbed and accumulated by plants and animals.
- In human body, ergothioneine can enter into the nucleus and mitochondria by transporter protein OCTN1 and plays a physiological role in scavenging free radicals, repairing cells, maintaining DNA homeostasis, stimulating cell self-vitality and inhibiting apoptosis.
- Ergothione is natural, safe and non-acne-causing. Many products that contain EGT as a main ingredient have been launched in European, the USA, and Japan, such as common food and oral beauty products.

Product Information

INCI Name: ERGOTHIONEINE

Other Names：ERGO；EGT

CAS No.: 497-30-3

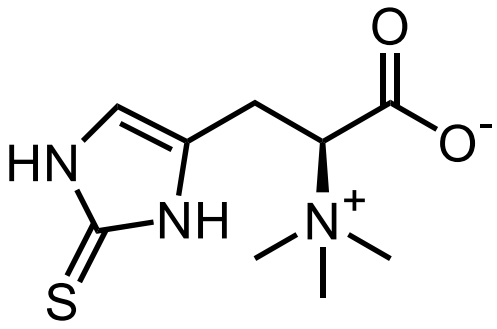
Appearance: White crystal

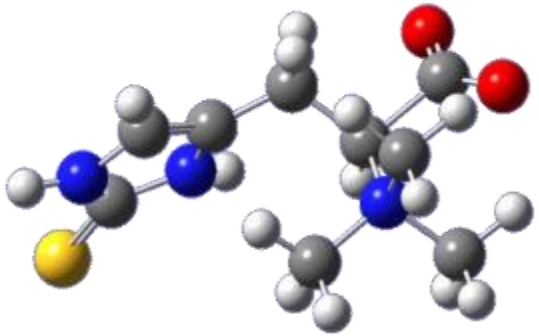
Melting point: 275-277 ℃

Optical rotation: $[\alpha]_D \geq (+)122°$（c=1, H_2O）

Suggested dosage: 0.1%-1.0%

Chemical Structure





01

Super Natural Antioxidant

02

Targeting Antioxidant Source - Mitochondria

03

Safe, Stable, Long-acting, and Recycled

04

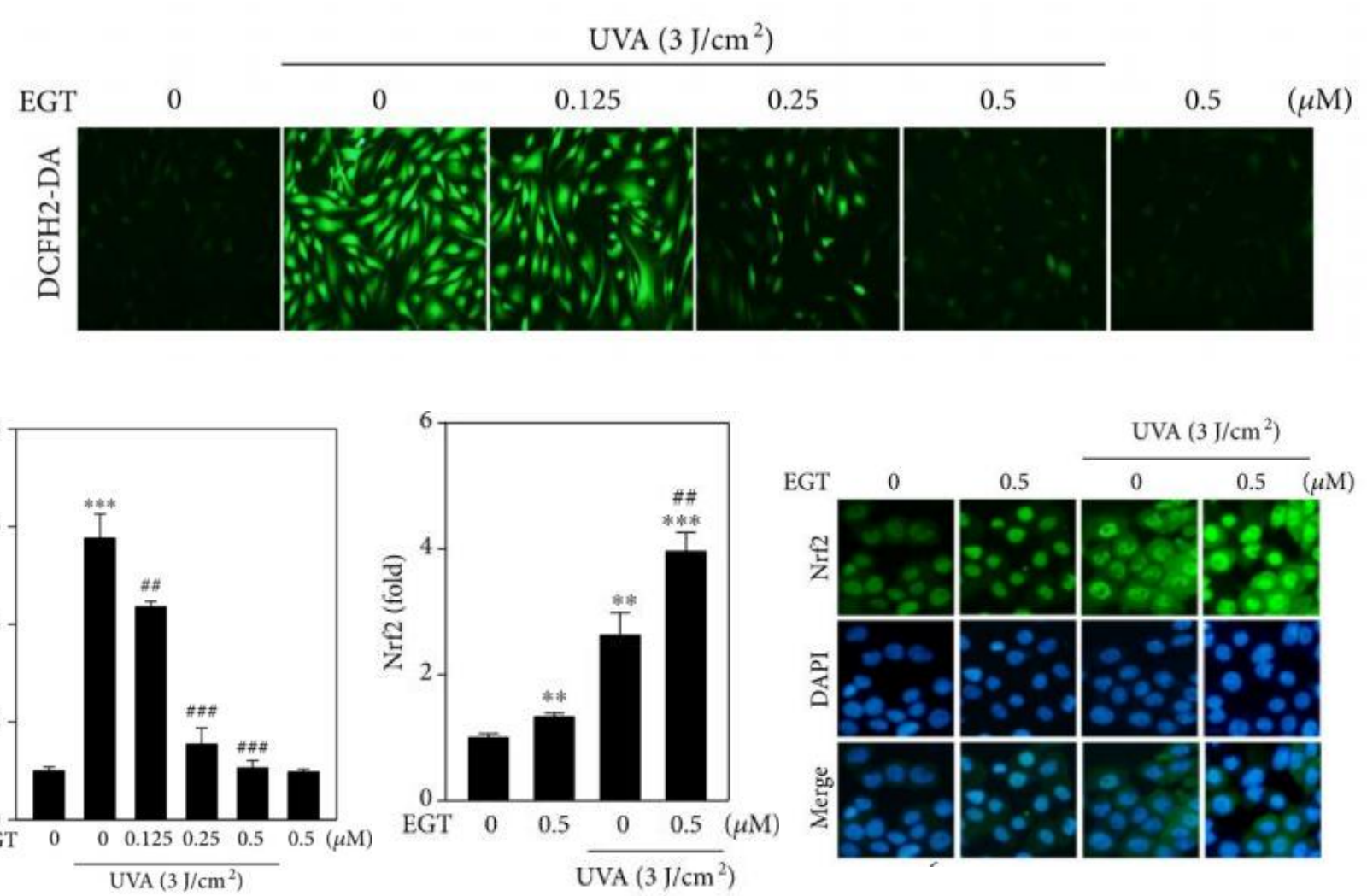
Super Synergistic Effect with Other Actives

Efficacy of Ergothioneine--Super Natural Antioxidant

Experiment of scavenging UVA-induced ROS

Experiment:
Use UVA to induce the ROS burst in HSF. Pretreatment HSF cells with EGT (0.125-0.5 μM) for 24 hours, then irradiate cells with 3 J/cm2 by UVA. DCF shows ROS levels inside the cells, observe the ergothioneine with a fluorescence microscope (200x magnification).

Conclusion:
Pretreat the cells with 0.13 ppm EGT solution，the removal rate of ROS is 90%.



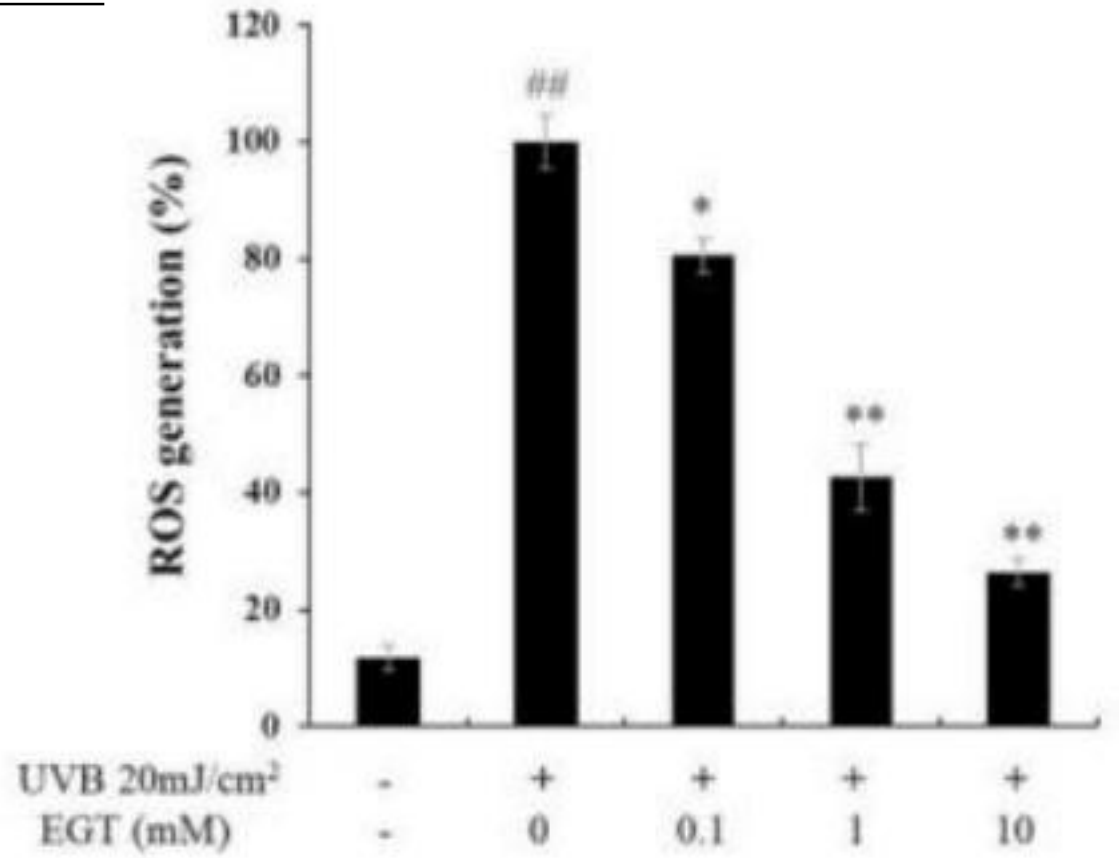
Oxidative Medicine and Cellular Longevity 2020(10):1-13

Efficacy of Ergothioneine--Super Natural Antioxidant

Experiment of scavenging UVB-induced ROS

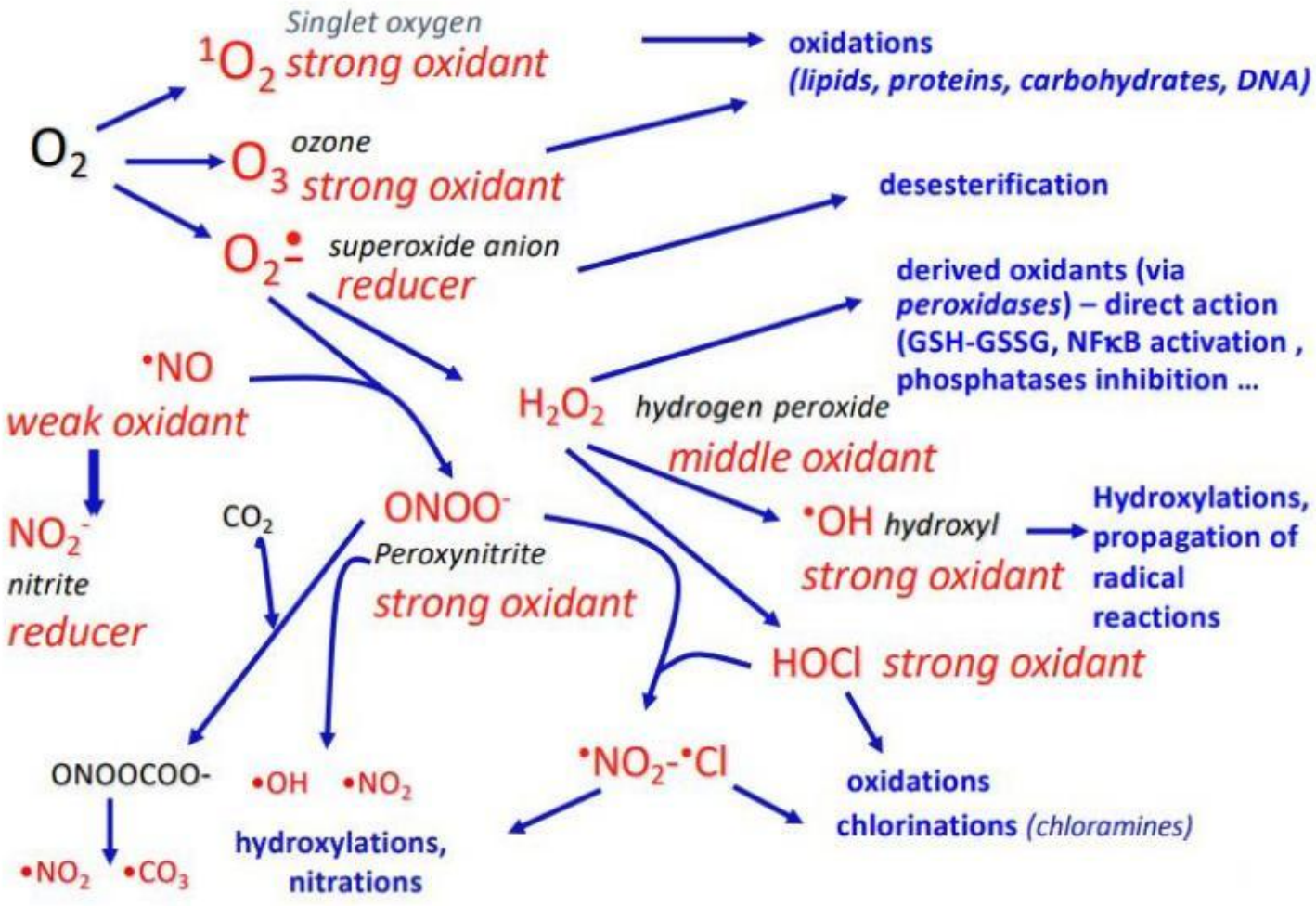
Experiment:
Pretreat Keratinocytes (HaCaT) with different concentrations of EGT culture for 2 hours, then change medium, expose to UVB (20 mJ/cm^2), and incubated with fibroblasts for 48 hours at 37°C.

Conclusion:
Pretreat the cells with 0.2% EGT solution , the removal rate of ROS is 75%.



Experimental Cell Research 400 (2021) 112516

Efficacy of Ergothioneine--Super Natural Antioxidant



FREE RADICALS

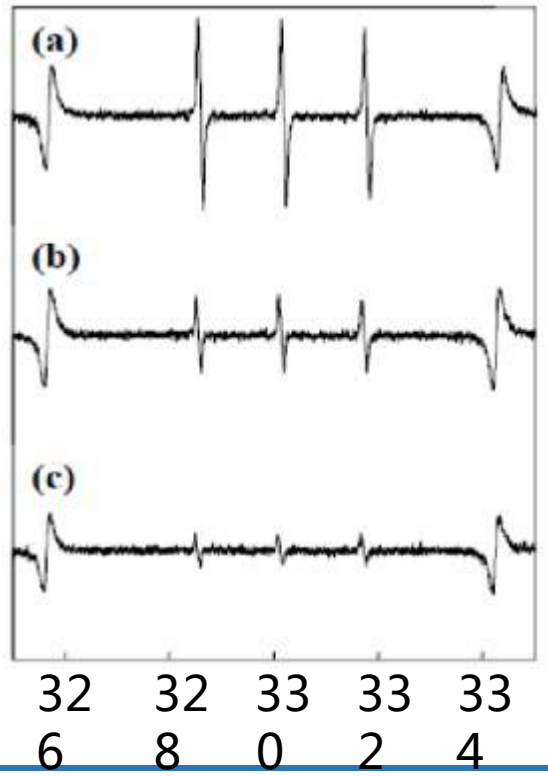
Free radicals can be divided into ROS (oxygen radicals), RNS (nitrogen radicals), RCS (lip oxygen radicals), RClS (chlorine radicals). EGT has a broad spectrum of scavenging free radicals.

Examples of free radicals

1. Superoxide anion radicals (O2)
2. Hydroxyl radicals (·OH)
3. Carboxyl free radicals (ROO·)
4. Lip oxygen radicals (RCIS)
5. Nitric oxide radicals (NO·)
6. Nitro radicals (·ONOO-)

Ability of scavenging ROS (singlet oxygen/superoxide anion)

The most common method for detecting reactive radicals is spin trapping method



Control

Singlet oxygen is highly active.

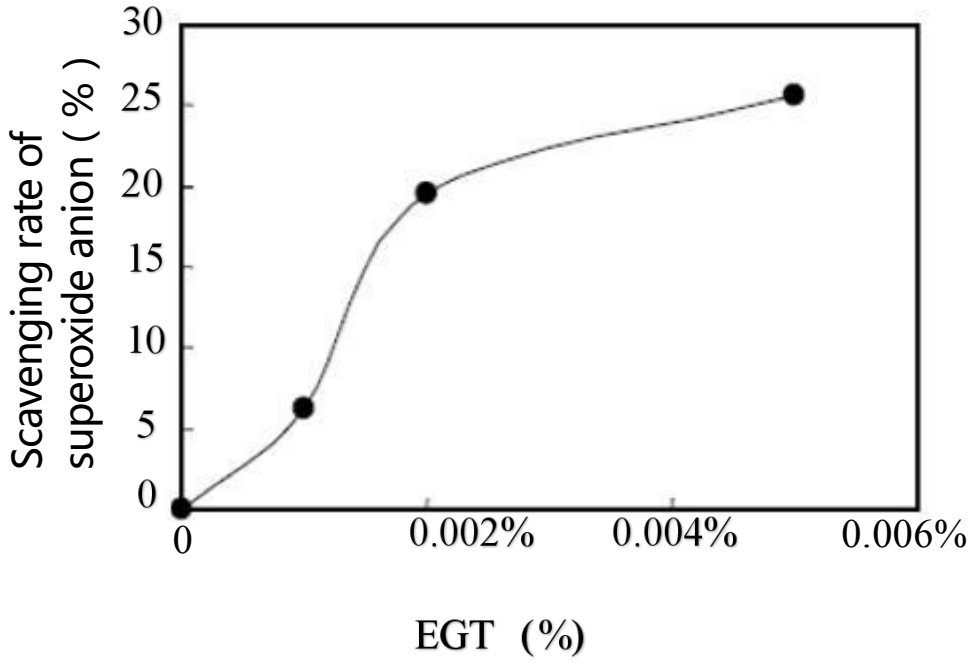
EGT 1%

EGT 2%

An approximately straight line indicates that Singlet oxygen is captured by EGT

Conclusion: 2% EGT can basically capture all singlet oxygen.

The ability of scavenging superoxide anions is the key indicator of antioxidant capacity



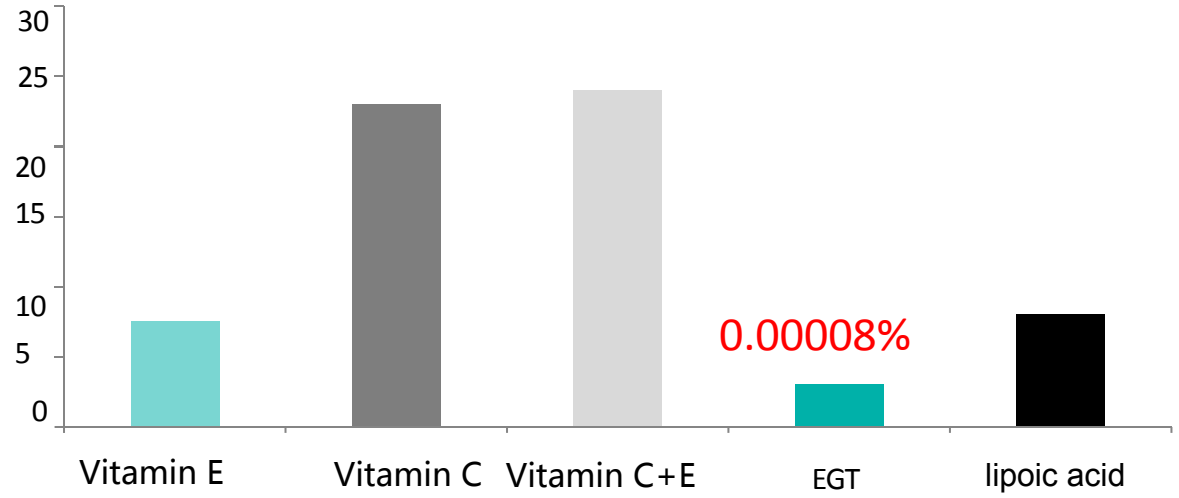
Conclusion: 0.005% EGT can remove 22% of superoxide anions.

————Obayashi et al., J. Cosmet. Sci. 56:17, 2005

Efficacy of Ergothioneine--Super Natural Antioxidant

Comparison experiment of scavenging Ozone

Conclusion:
The IC50 value of EGT to scavenge ozone is 0.8 μg/ml, which is only 1/10 of lipoic acid.



Comparison experiment of scavenging hydroxyl radical （-OH）

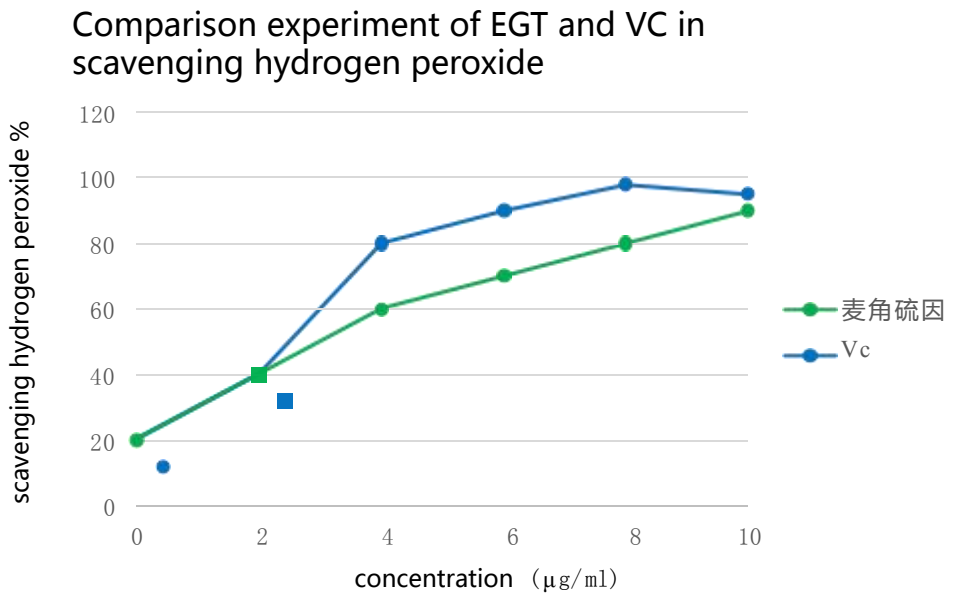
Conclusion:
When the concentration reaches 100 μg/ml, the scavenging rate of hydroxyl radical of ergothione is 96.71% and the VC is 86.28%

Test data from Joint Lab of Shanghai Institute of Technology He et al., Skin Pharm. Phys. 17:183, 2004

Efficacy of Ergothioneine--Super Natural Antioxidant

ANTI-AGING

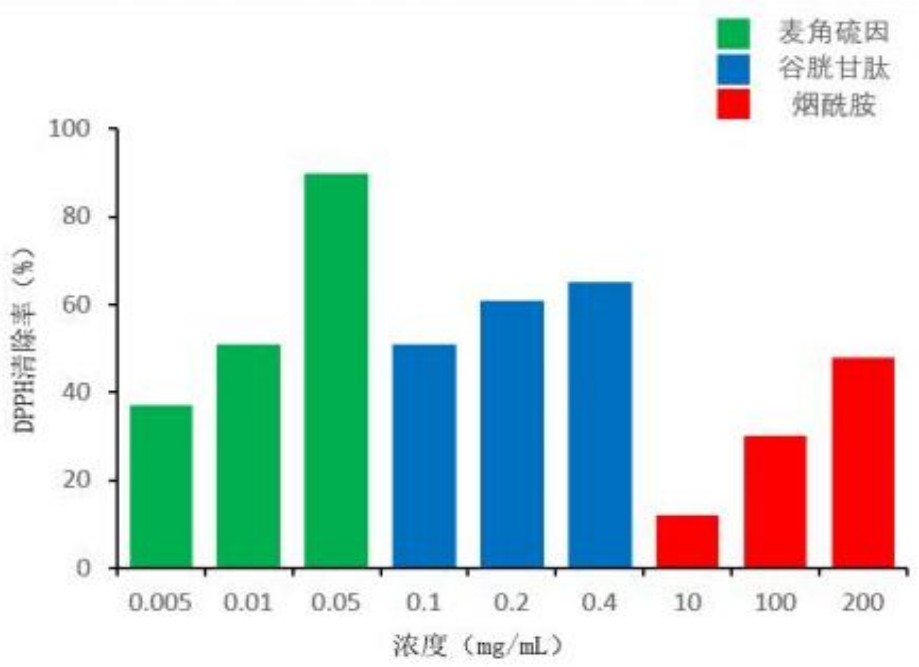
Comparison experiment of scavenging hydrogen peroxide



Conclusion:

The IC50 value of EGT to scavenge hydrogen peroxide is 3.03 μg/ml，and the VC is 1.69 μg/ml. When the EGT concentration reaches 10 μg/ml, the hydroxyl radical scavenging rate is 90.51%，which is comparable to Vc.

Comparison experiment of scavenging DPPH



Conclusion:

EGT has a good DPPH scavenging effect, and The IC50 value of EGT is 7.88 μg/mL, which has an great antioxidation advantage over glutathione and nicotinamide. When the concentration of EGT reaches 50μg/mL, the DPPH scavenging rate is more than 90%.

Test data from Joint Lab of Shanghai Institute of Technology

Efficacy of Ergothioneine--Super Natural Antioxidant

Ability of scavenging RNS (ONOO-)

Superoxide radicals (O2-) and nitric oxide (NO.) combine very rapidly to form peroxynitrite (ONOO-)

Antioxidant Action of Ergothioneine: Assessment of Its Ability to Scavenge Peroxynitrite

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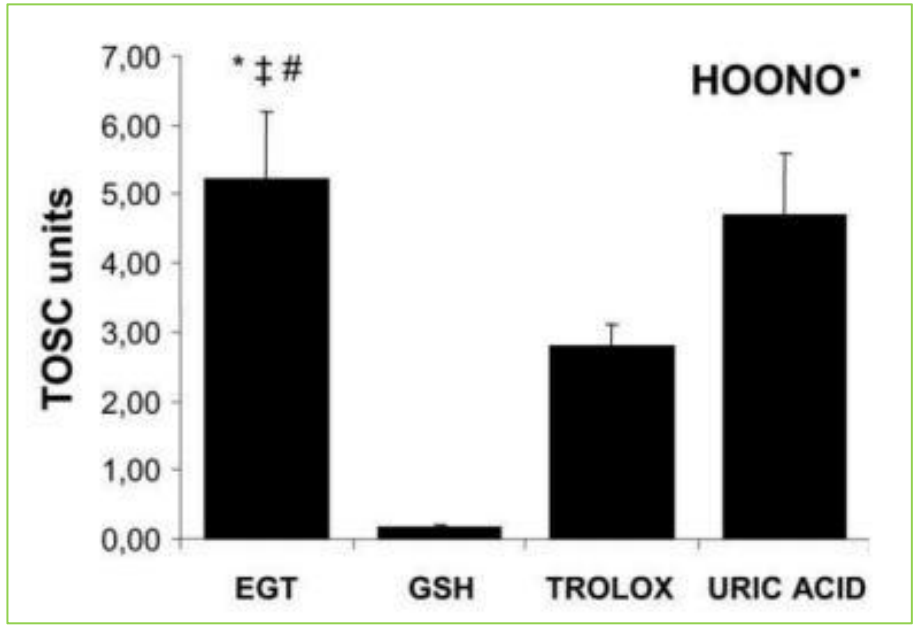
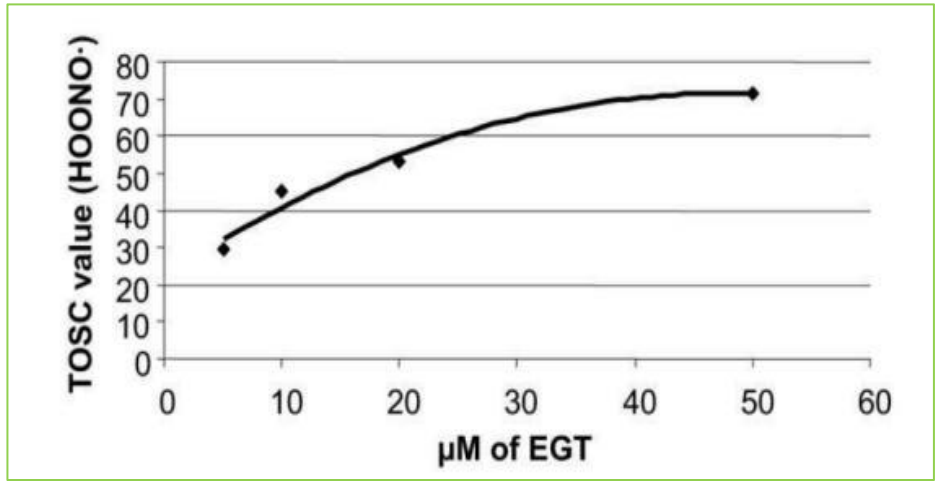
Received January 2, 1997

The superoxide radical ($O_2^{\cdot}$) and nitric oxide ($NO^{\cdot}$) combine very rapidly to form peroxynitrite ($ONOO^-$), a reactive tissue damaging nitrogen species thought to be involved in the pathology of several chronic diseases. The natural product ergothioneine protects against the nitration of tyrosine and the inactivation of α_1-antiproteinase by $ONOO^-$. Ergothioneine merits further investigation as a biological and therapeutic antioxidant agent. © 1997 Academic Press

radioprotective effects [25], scavenge singlet oxygen [28], scavenge HOCl and hydroxyl radicals [29,30], possess antimutagenic properties [31] and to scavenge peroxyl radicals -with a calculated rate constant for reaction with the model radical trichloromethylperoxyl ($CCl_3O_2^{\cdot}$) of 1.2×10^9 $M^{-1}s^{-1}$ [32]. Ergothioneine has been been linked to the metabolism of iron, copper and zinc [25,33] and inhibition of metalloenzymes [34].

In this paper, we show that ergothioneine is a powerful scavenger of $ONOO^-$ able to protect α_1-antiproteinase against inactivation, and tyrosine against nitration, by $ONOO^-$.





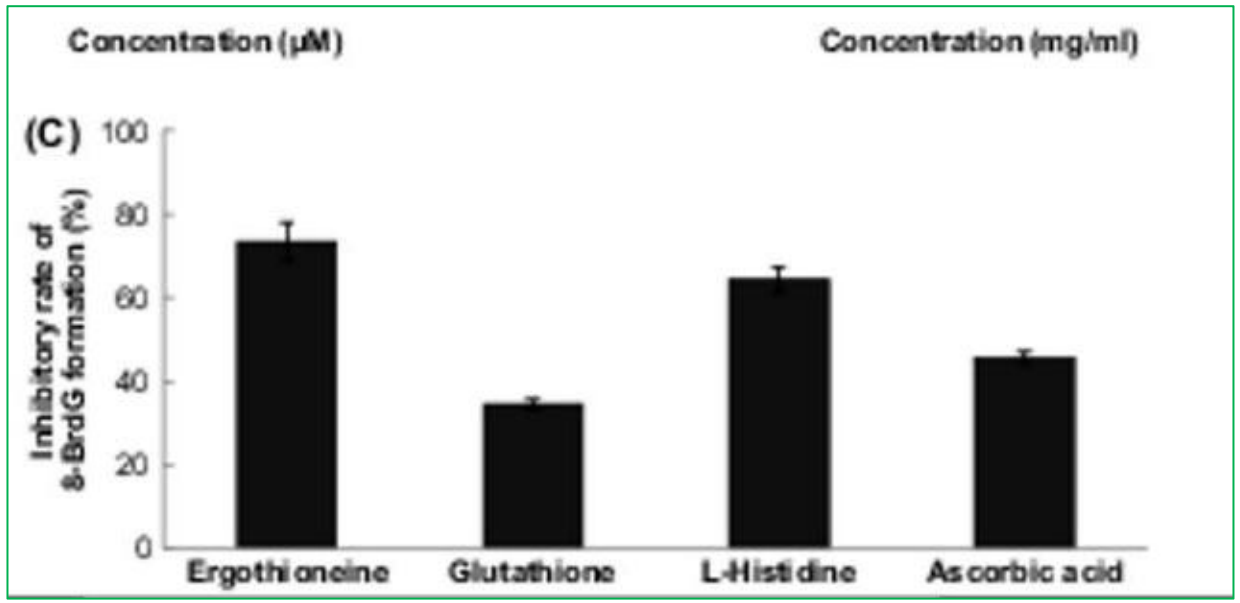
Conclusion:

Ergothioneine is a powerful scavenger of ONOO-. At a concentration of 0.00125%, the scavengering rate of ONOO-can reach 68%. Protects α_1-antiprotease from inactivation and protects tyrosine from nitrification, thereby could protect hyaluronic acid, fibronectin, elastin, etc.

Biochem Biophys Res Commun. 1997 Feb 13;231(2):389-91.

Ability of scavenging RCIS (hypochlorous acid)

Conclusion:
Ergothioneine is a powerful scavenger of RCIS and could protect α_1-antiprotease.



Efficacy of EGT as a HOBr scavenger

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Review

Ergothioneine; antioxidant potential, physiological function and role in disease ☆

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The highly reactive ferryl-hemoglobin, derived from oxidation of oxyhemoglobin, plays a critical role in lipid peroxidation in erythrocytes [62]. As previously mentioned EGT was able to reduce ferryl-myoglobin/hemoglobin and also prevented the peroxidation of arachidonic acid by a mixture of H_2O_2 and heme protein. Furthermore, Spicer et al. [96] found that the rate of nitrite-induced oxidation of hemoglobin in isolated rabbit blood, to methemoglobin, was inversely proportional to EGT content, and subsequent addition of EGT reduced methemoglobin back to hemoglobin. Thus EGT may act as a protectant against peroxidation in blood [58]. Correspondingly, dietary intake of EGT was shown to retard nitrite-induced methemoglobin formation in rabbits [96]. The ability of EGT to scavenge HOCl [58], may also be relevant in protecting erythrocytes from damage by neutrophils, the principal source of HOCl in the body [62].

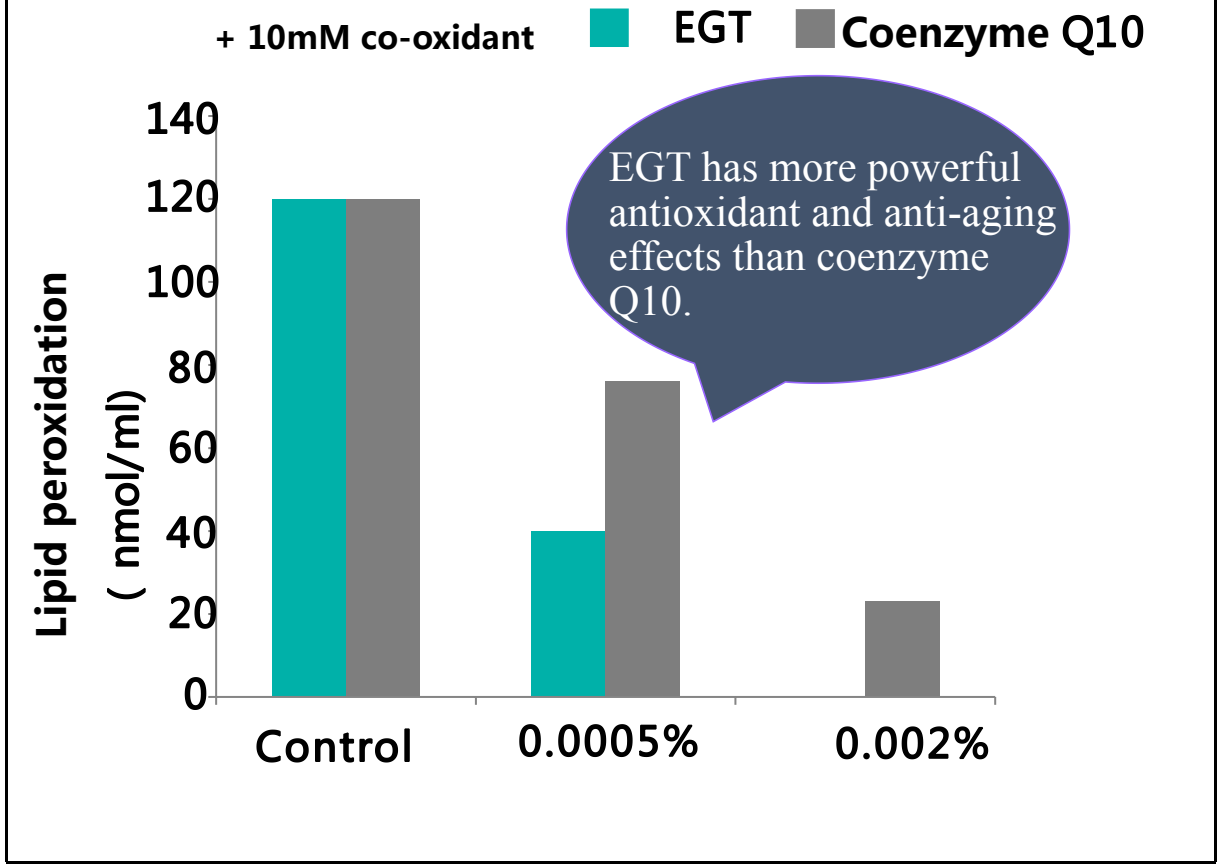
Arch Biochem Biophys. 1991 Jul;288(1):10-6.
Bioscience, Biotechnology, and Biochemistry, Volume 80, Issue 2, 1 February 2016, Pages 313–317

Efficacy of Ergothioneine--Super Natural Antioxidant

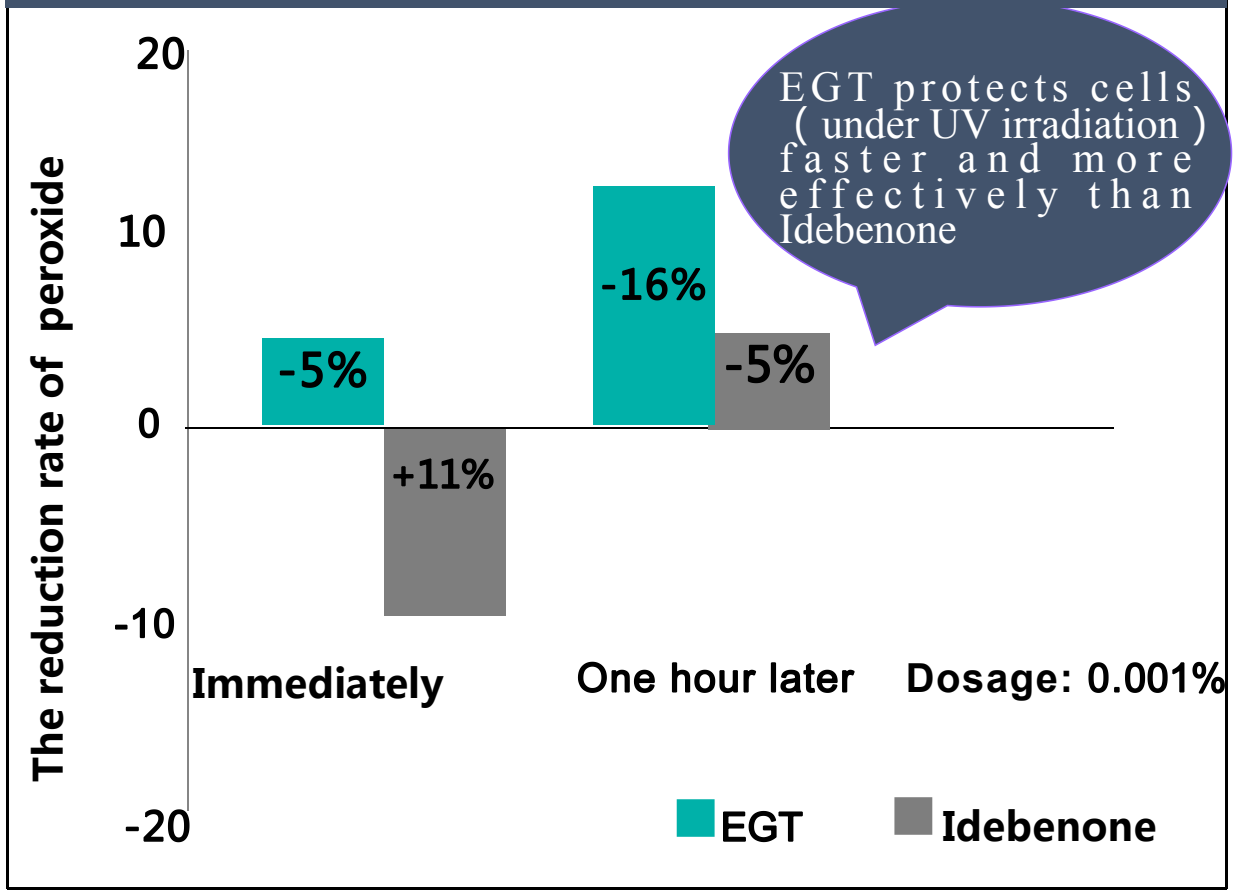
ANTI-AGING

Comparing antioxidant capacity with coenzyme Q10 and Idebenone

EGT can stop peroxidation at 0.002%, but coenzyme Q10 can’ t.

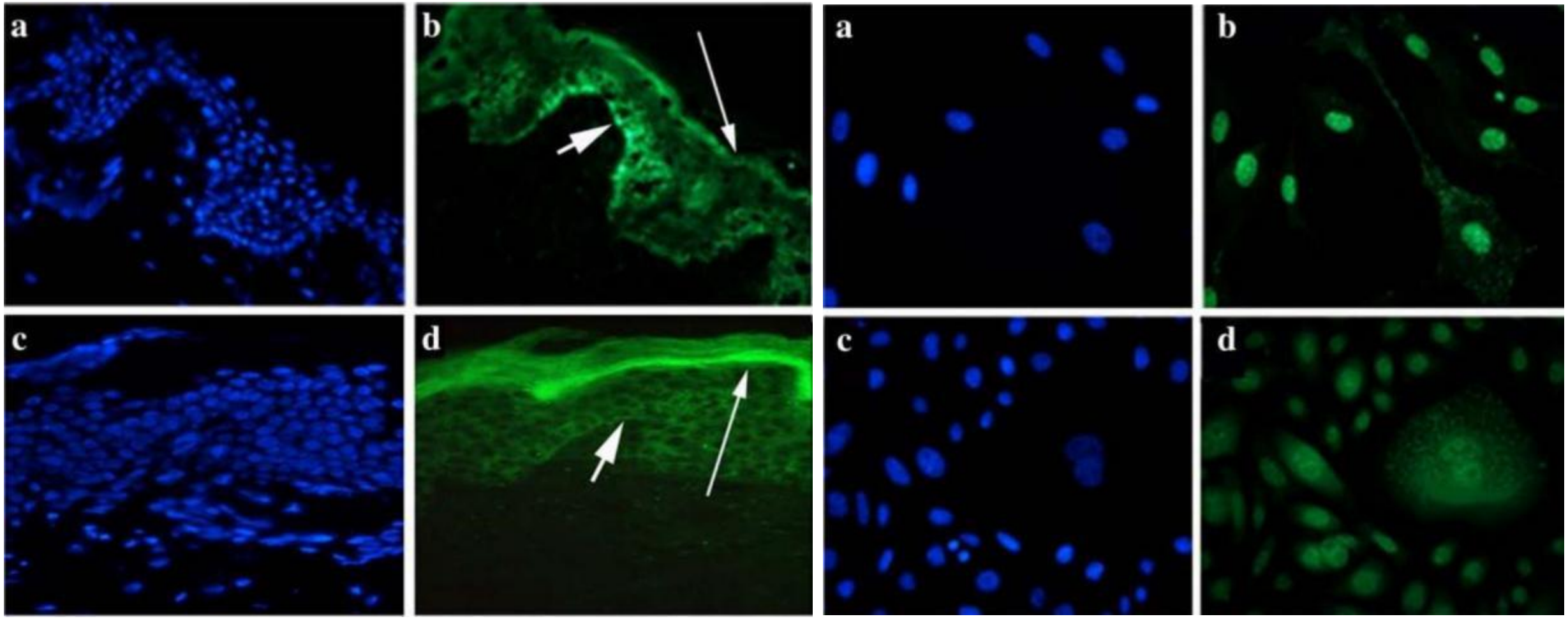


The reduction rate of peroxide in EGT-containing cells is 16% , which is 3 times of Idebenone.



Dong, Kelly K., et al. 2007

Distribution of EGT transporter protein OCTN-1 in skin cells



OCTN1 protein in adult skin (left b), pediatric foreskin skin (left d), adult dermal fibroblasts (right b)， adult keratinocytes (right d)

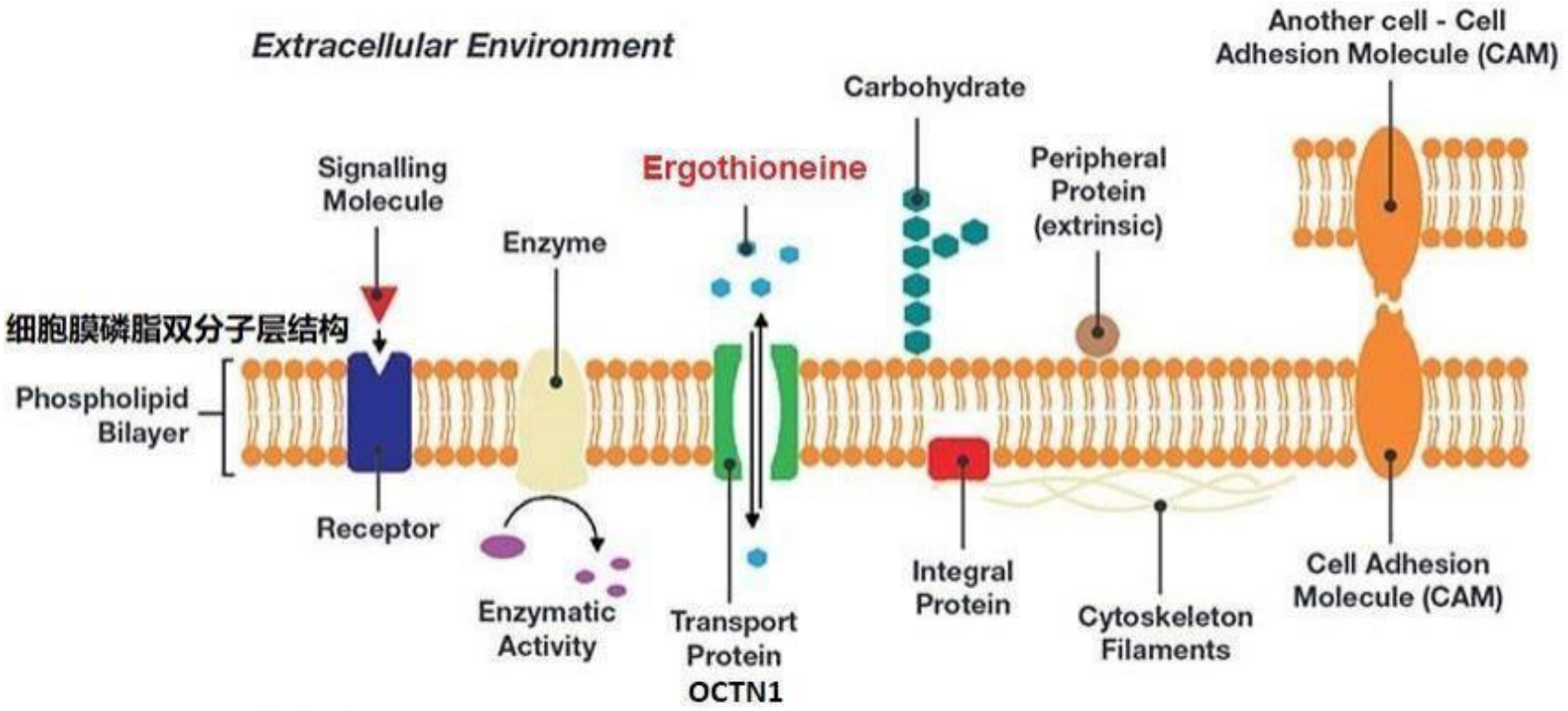
Note: Blue is the nucleus, green is OCTN1

N.G. Markova et al. / Free Radical Biology & Medicine 46 (2009) 1168– 1176

Exclusive tranporter protein OCTN1 on biofilms

The human body cannot synthesize EGT by ourselves, and needs to ingest with diet. EGT is water-soluble, but our cell membrane is phospholipid bilayer, How can water-soluble substance permeate lipid-soluble substance?

EGT has an exclusive tranporter, protein OCTN1 on human cells membrane, coding for gene SLC22A4. OCTN1 can transport the EGT into cell, mitochondrial and nucleus.

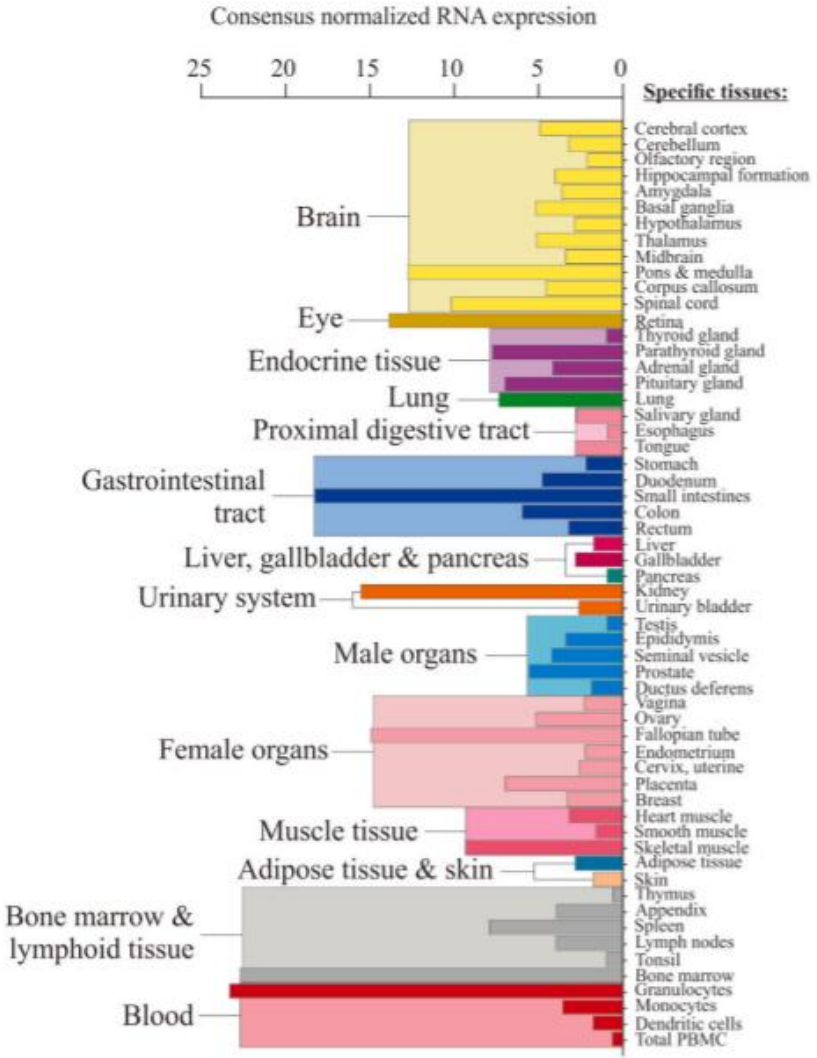


(Gründemann, D. H., Discovery of the Ergothioneine Transporter. Proc. Natl. Acad. Science (PNAS) (2005), 102(14), 5256-5261).

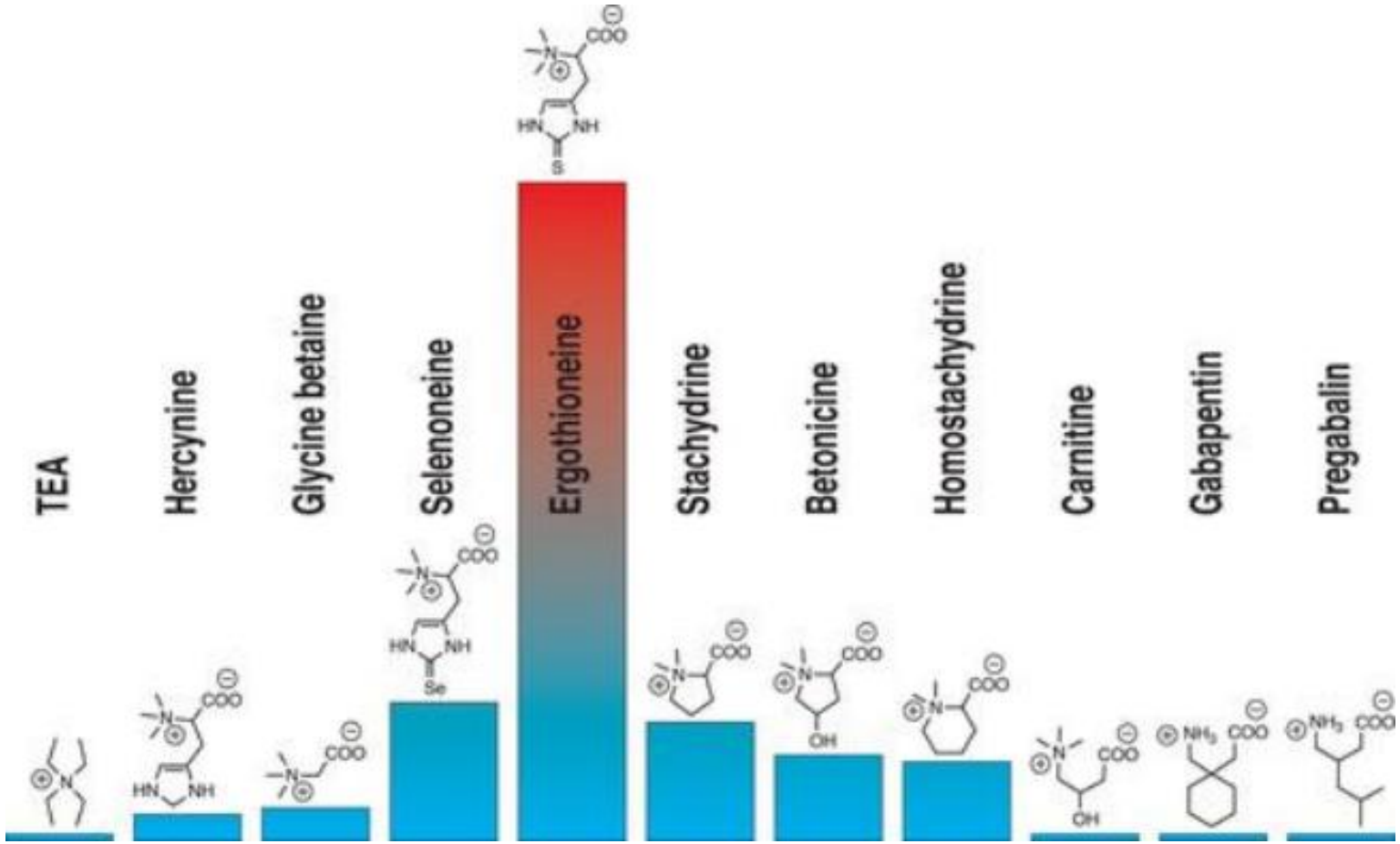
Efficacy of Ergothioneine--Mechanism of How EGT Affect our Body

ANTI-AGING

Stable distribution of OCTN1 in human cells



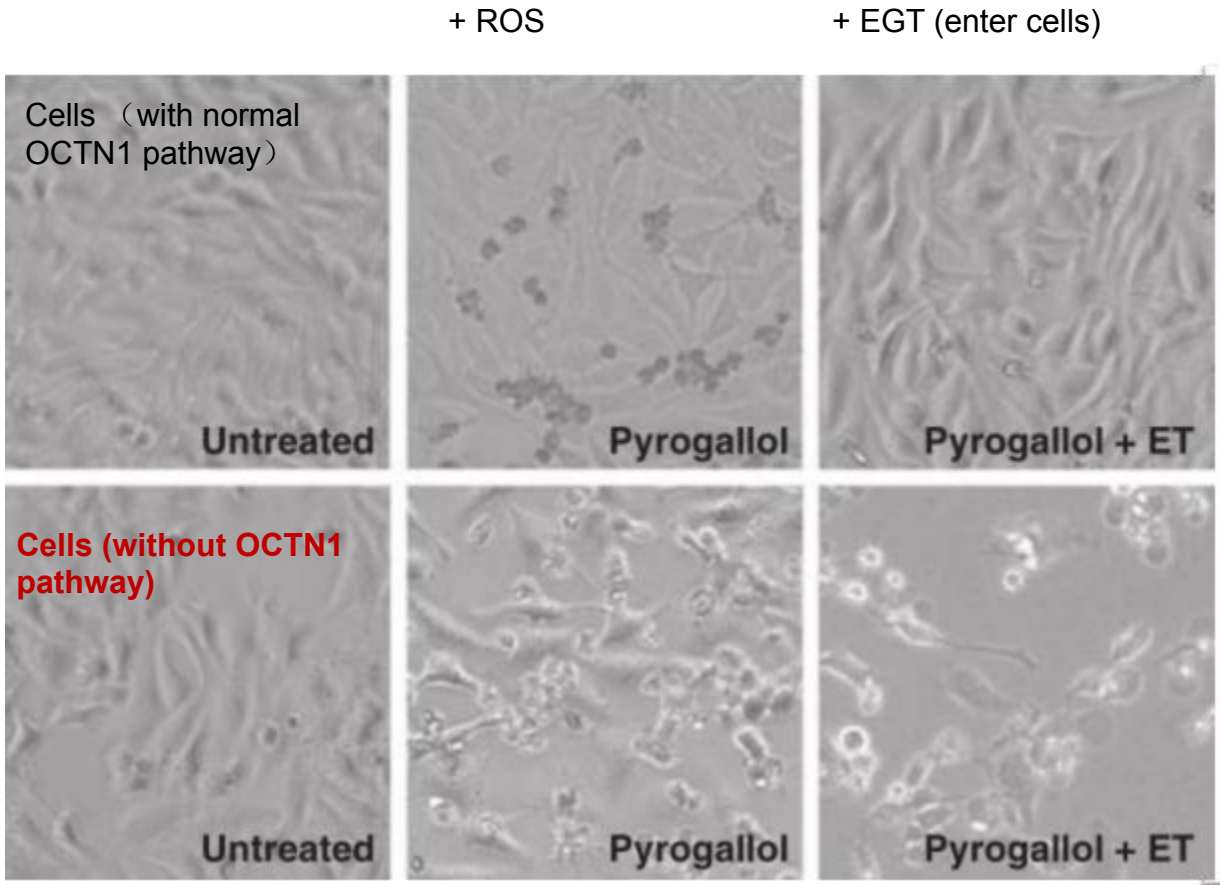
OCTN1 is the exclusive transporter of EGT

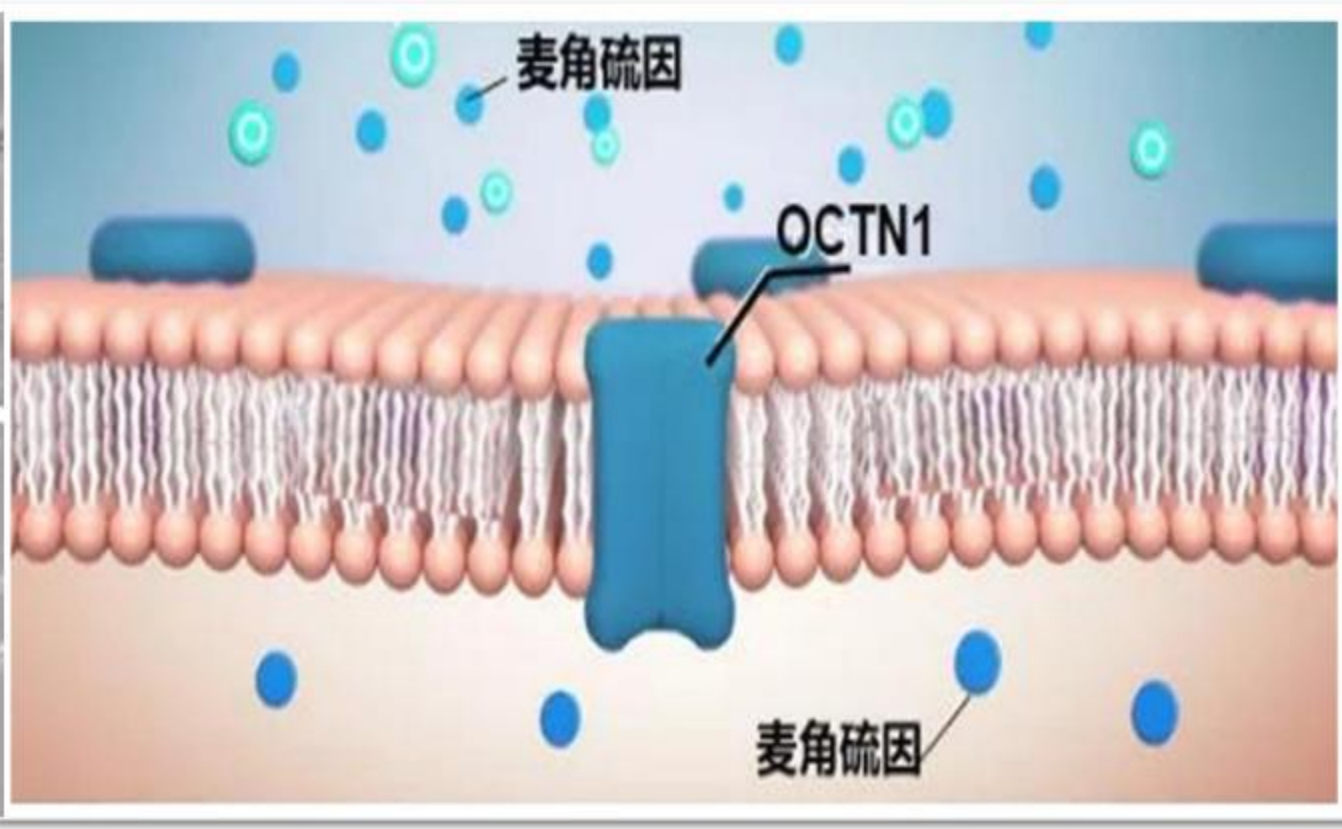


Redox Biology(IF 11.799) Pub Date 26 ,DOI:10.1016/j.redox.2021.101868
FEBS Letters (IF 4.124) Pub Date : 2021-12- 27 ,DOI:10.1002/1873-3468.14269

Efficacy of Ergothioneine--Mechanism of How EGT Affect our Body

ANTI-AGING





Experiment:

Hela cells, cultured with 1 mM EGT solution, treated with 150 μM (micron) catechol for 16 h.

Cells with normal OCTN1 maintain normal cell structure; Cells without OCTN1 pathway undergo severe morphological changes and result in apoptosis.

The unusual amino acid L-ergothioneine is a physiologic cytoprotectantCell Death and Differentiation (2010) 17, 1134– 1140

Efficacy of Ergothioneine--Targeting antioxidant source - Mitochondria

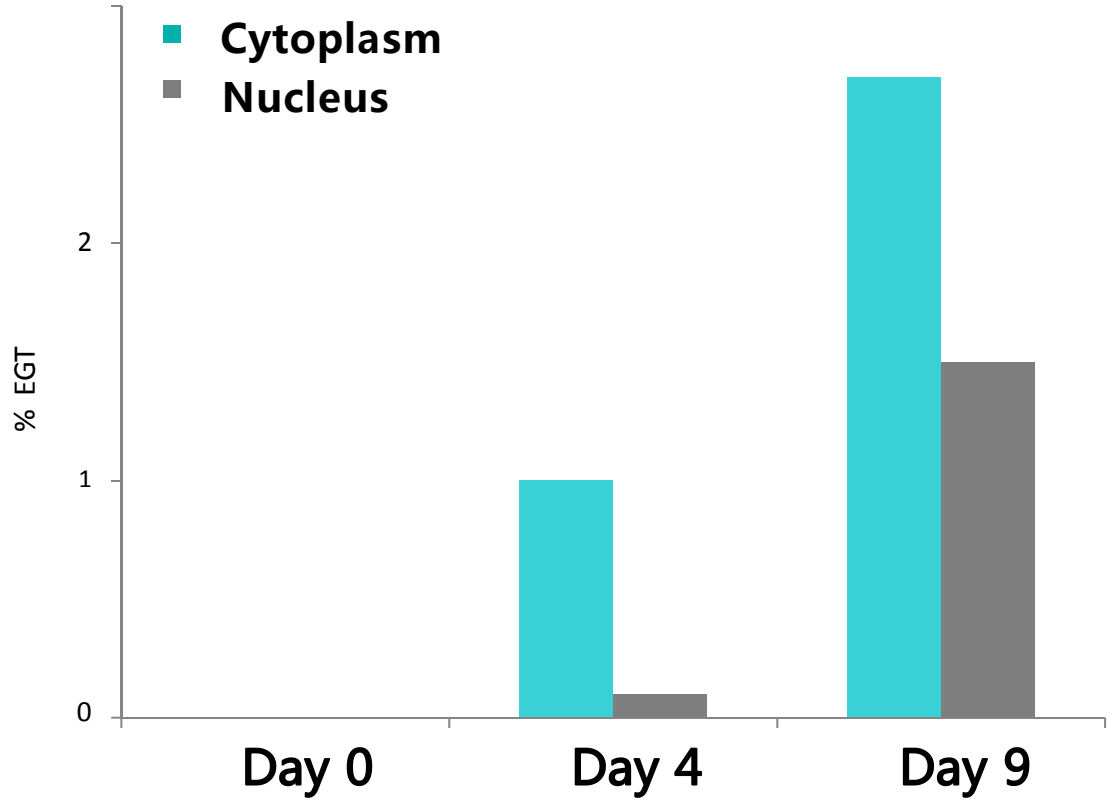
EGT accumulation experiment

Conclusion:

By the exclusive transporter OCTN1, EGT can penetrate the cell membrane and nuclear membrane, and can be accumulated in cytoplasm and nucleus.

Experiment：

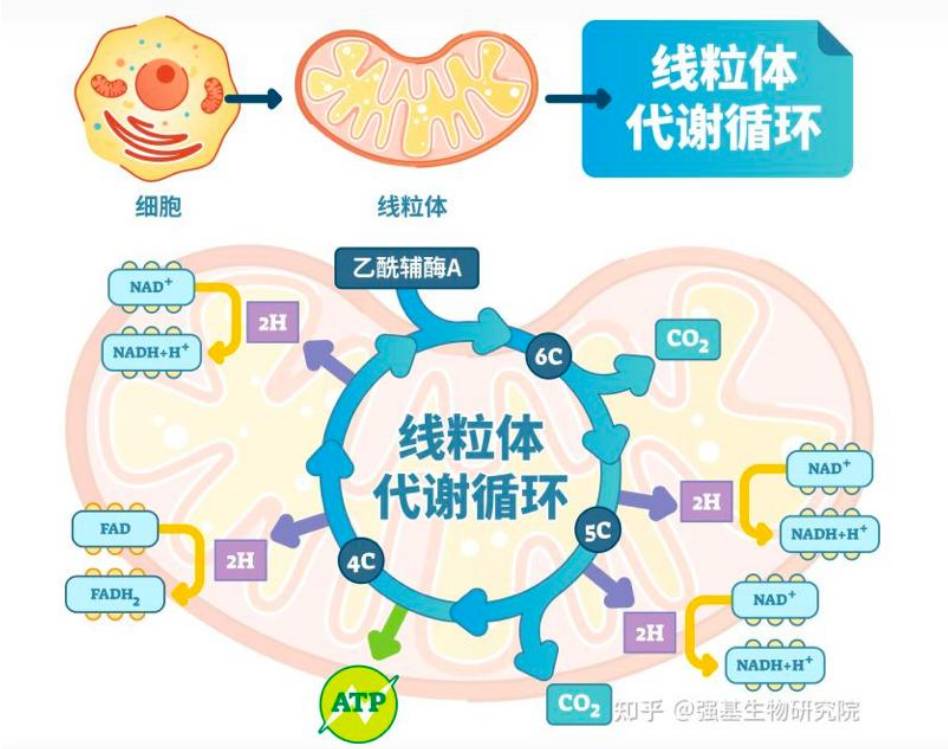
Supply the cells with 0.5% EGT culture continuously for 9 days, and analyse the accumulation of EGT in cytoplasm and nucleus.



N.G. Markova et al. / Free Radical Biology & Medicine 46 (2009) 1168– 1176

Oxidation reaction source -- Mitochondria

- Mitochondria are the energy factory / power plant of the cell. In human body, more than 90% of oxygen is consumed within the mitochondria, from where a large number of free radicals are generated.
- Whether mitochondrial is healthy is closely related the cell apoptosis. While providing energy ATP, mitochondria are also involved in other cellular activities, such as they can control the signal transmission between cells, cell differentiation and life and death cycle, etc.. If the mitochondria does not work properly, then it will release signals to prompt the cell to initiate the apoptosis program.



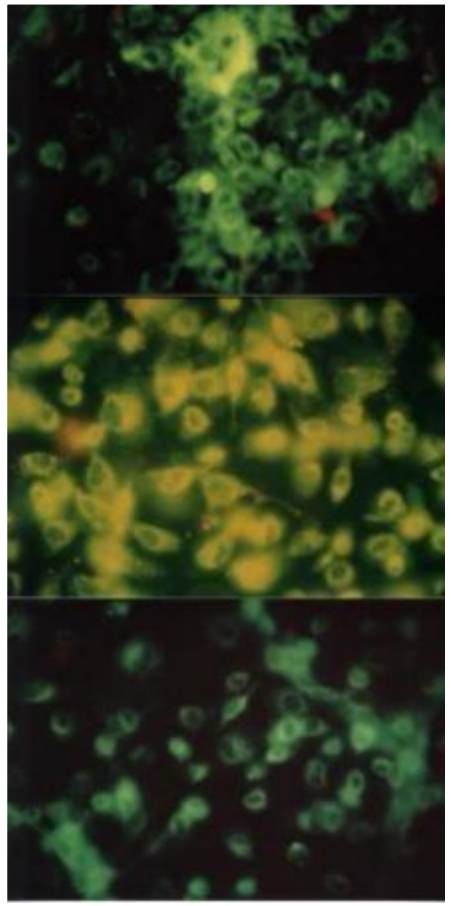
Boveris, A., Oshino, N., & Chance, B. (1972). The cellular production of hydrogen peroxide. Biochemical Journal, 128(3), 617–630.

Efficacy of Ergothioneine--Targeting Antioxidant Source - Mitochondria

EGT Antioxidant experiment of protecting Mitochondrial

Conclusion:

- Protein OCTN1 also exists on the mitochondrial membrane.
- EGT, as a super antioxidant, is the only antioxidant with a clear mechanism to repair mitochondria.



Keratinocytes shows green when working with normal mitochondria.

After the cells were treated with the biological agent alloxan, a large amount of ROS were released to kill mitochondria, and the cells turned yellow.

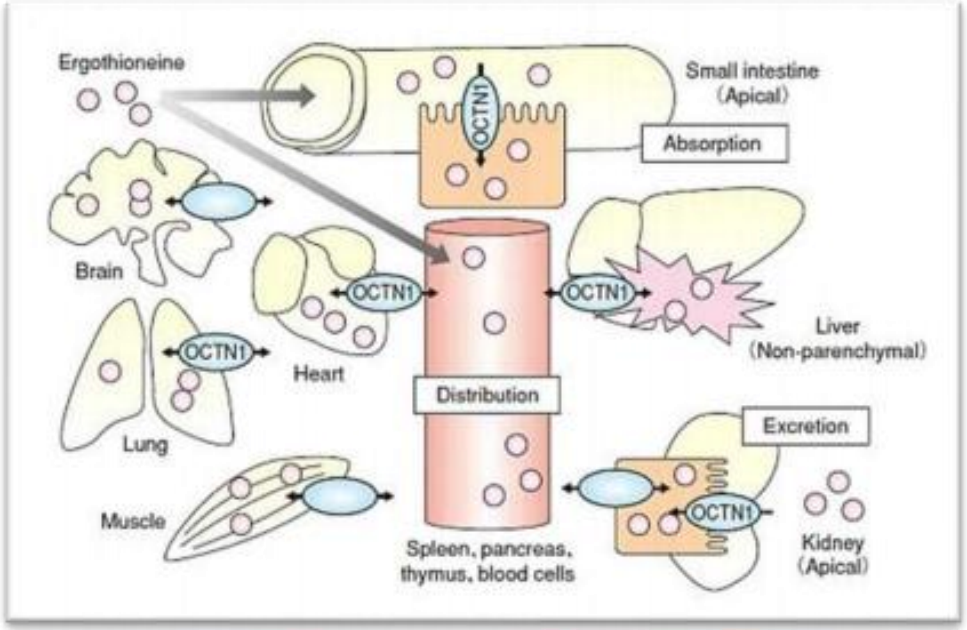
If simultaneous treatment with EGT and Alloxan, mitochondria were effectively protected,which indicated that free radicals were scavenged and the cells were protected.

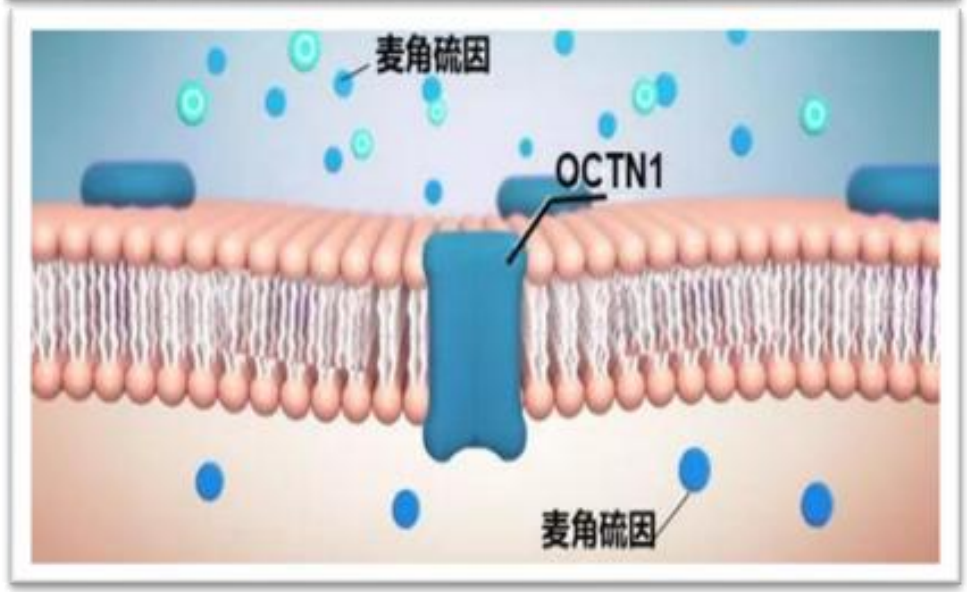
Efficacy of Ergothioneine--Mechanism of How EGT affect our body

ANTI-AGING

EGT accumulates in cells normally subjected to the levels of oxidative stress

- EGT is a water-soluble amino acid molecule that does not enter cells initiatively to interfere with the balance of free radicals. When there are excessive free radicals in the cells, EGT will be passively transported into the organelle of the desired cells through the exclusive transporter protein OCTN1.
- Protein OCTN1 is encoded by gene SLC22A4 in mammals and distributes in small amounts on the surface of normal cell biofilms. When there is oxidative stress in the cells, the cells will upregulate gene SLC22A4, and then accumulate more and more protein OCTN1 on the biofilm (such as cell membrane, nuclear membrane, mitochondrial membrane) to transport EGT into cells and organelles to remove excessive free radicals; When the cells return to normal state, the cells will downregulate gene SLC22A4, and let the ROS in the cells restore to normal state.
- Our human body cannot synthesize EGT and need to ingest from outside world. EGT mainly comes from fungi such as mushrooms, matsutake, etc.

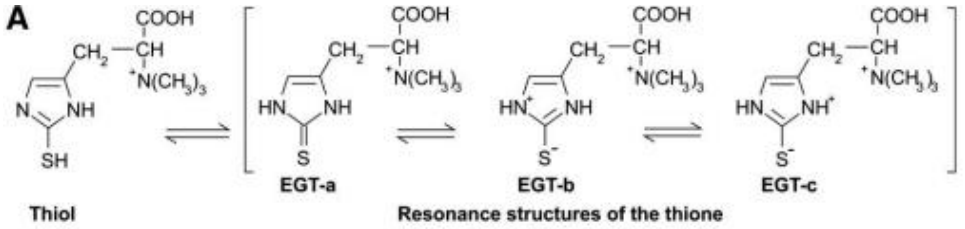


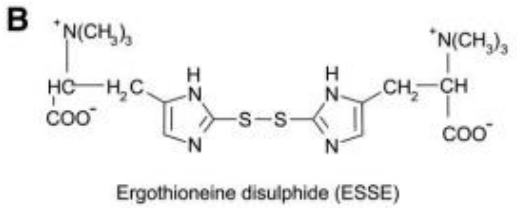


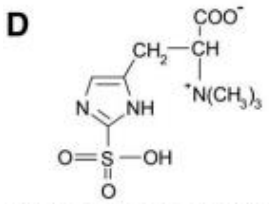
PNAS(IF 11.205) Pub Date : 2005-03-28 , DOI: 10.1073/pnas.0408624102

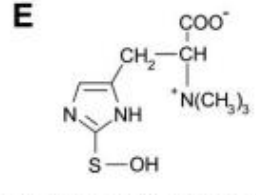
Efficacy of Ergothioneine--Safe, Stable, Long-acting, and Recycled

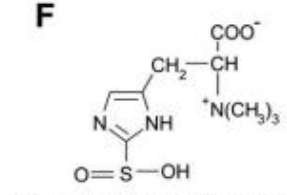
ANTI-AGING

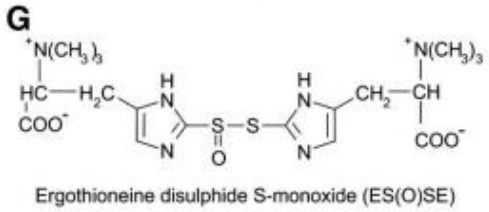


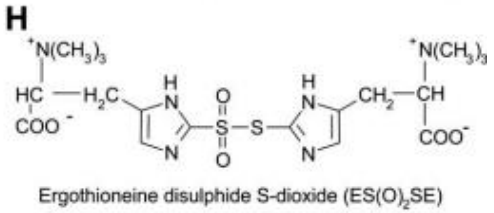


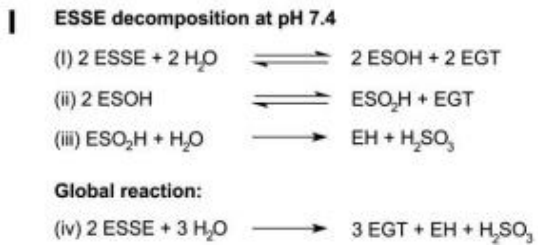


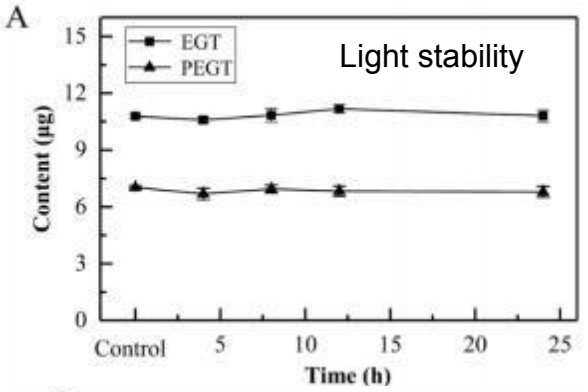


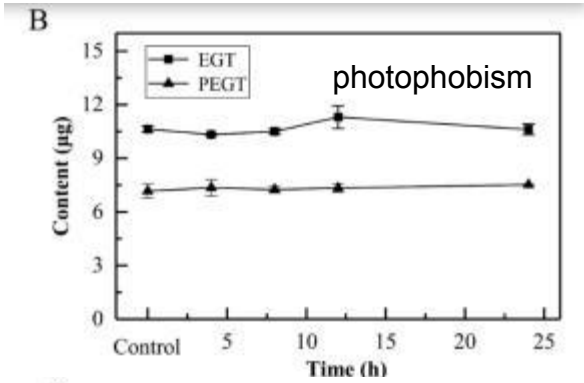


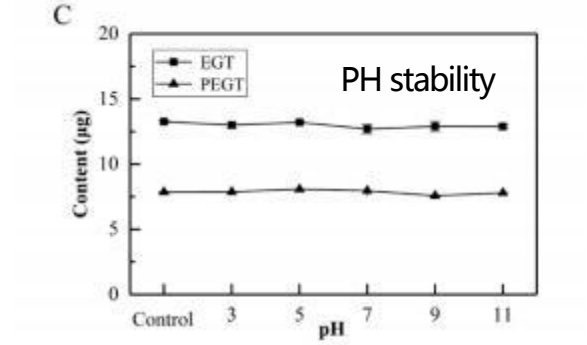


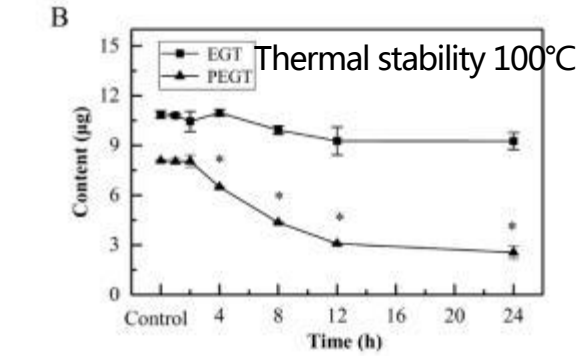












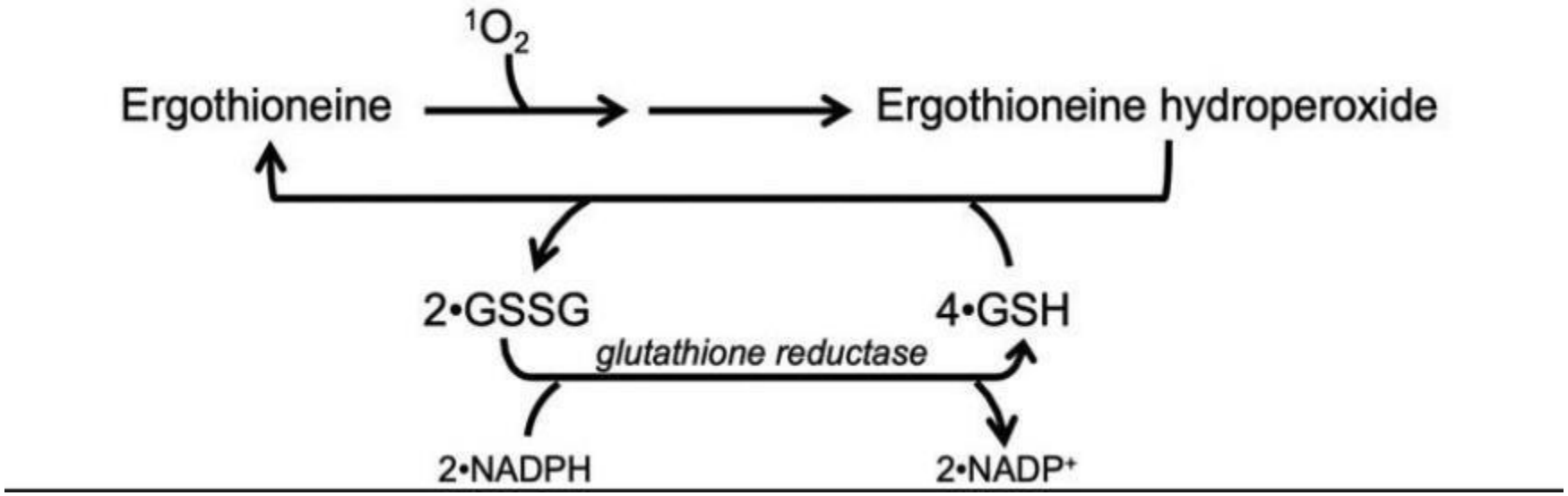
Conclusion:

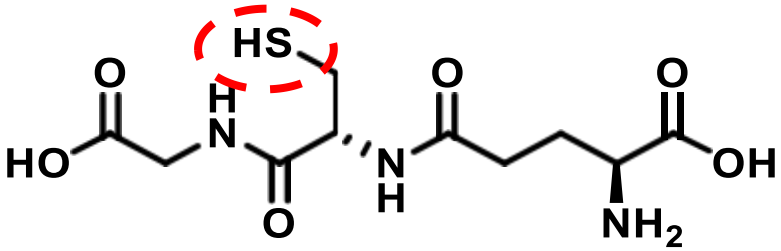
- EGT is stable: stable to light, heat and different PH.
- EGT is safety. Its half-life in vivo is 730 hours. EGT exists stably in the form of thione (A) in human body and is not involved in liver and kidney metabolism. Excessive intake of EGT will be converted into sulfate （D） which will be excreted through the urinary system.

Ergothioneine; Antioxidant Potential, Physiological Function and Role in Disease. *Biochim. Biophys. Acta* 1822, 784–793
International Journal of Medicinal Mushrooms, 22(3):211 – 220 (2020)

Efficacy of Ergothioneine--Safe, Stable, Long-acting, and Recycled

Recycled pathway of EGT in vivo

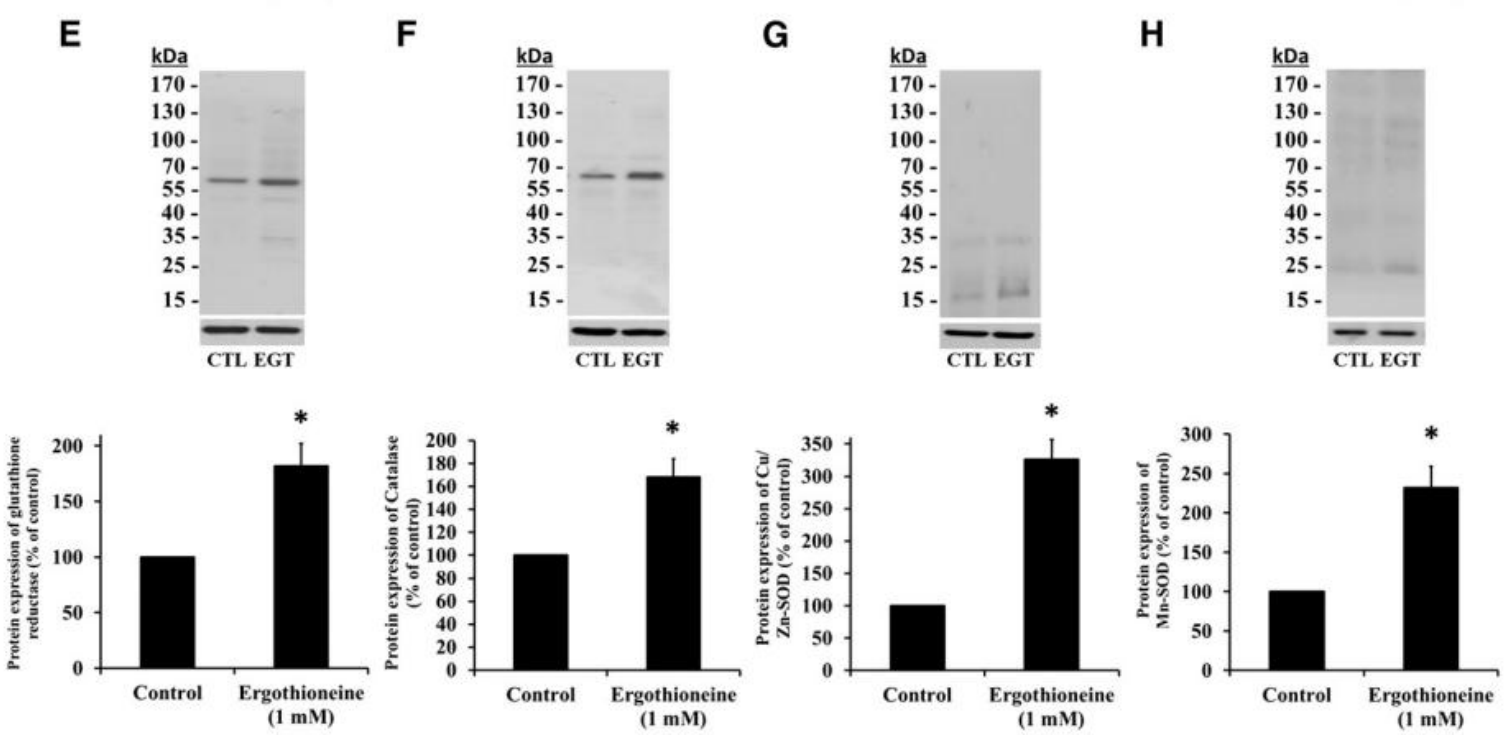




Glutathione（GSH）

Free Radical Biology & Medicine, 23 Oct 2017, 113:385-394
FEBS Letters, 26 December 2021, DOI: (10.1002/1873-3468.14268)

Efficacy of Ergothioneine--Super Synergistic Effect



(E) glutathione reductase, (F) catalase, (G) Zn/Cu-superoxide dismutase, (H) Mn-SOD

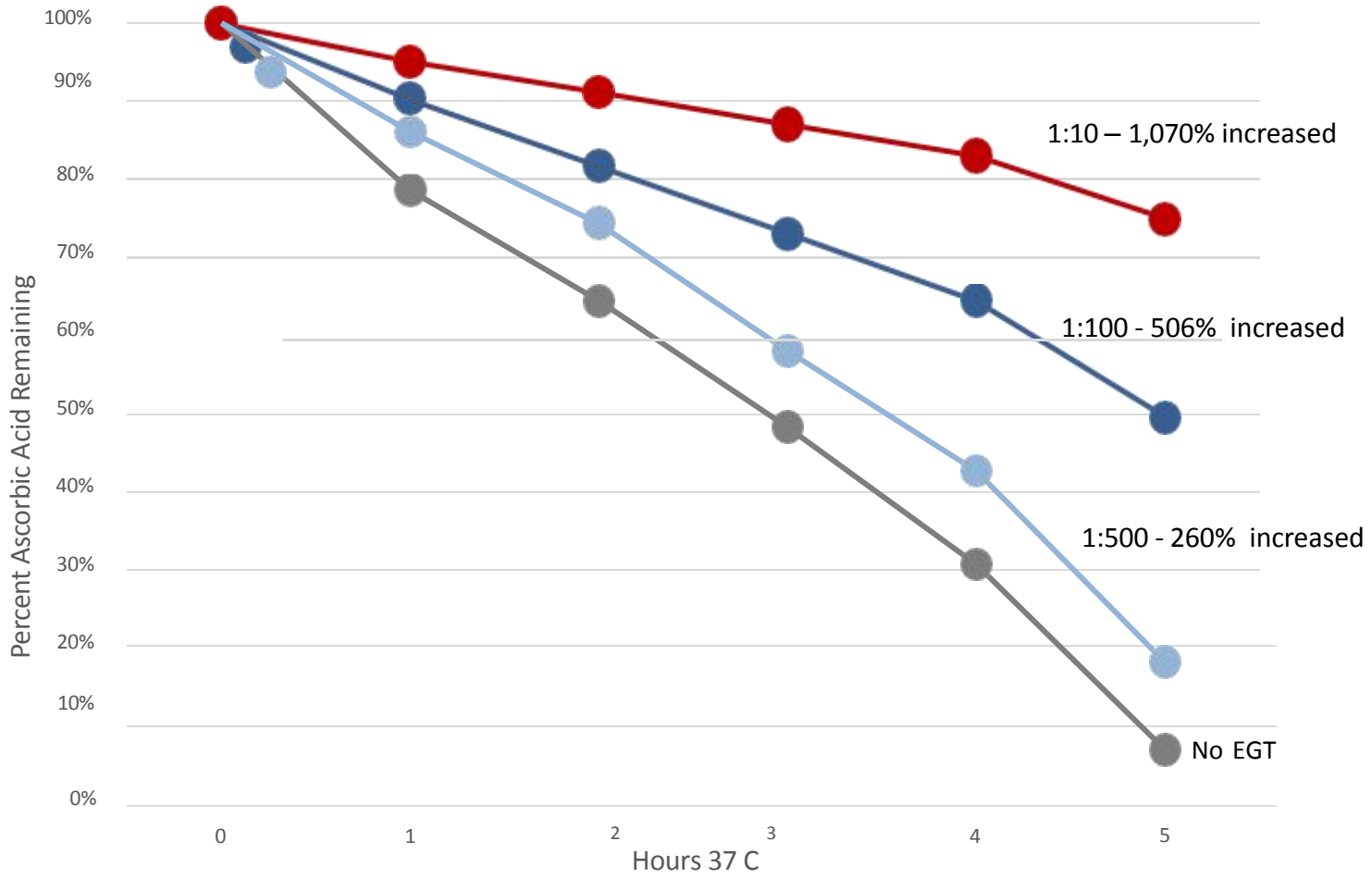
Synergistic effect with antioxidant enzymes

Cooperate synergisticly with glutathione reductase (GR), CAT and Zn-SOD, Cu-SOD, Mn-SO.

Experiment:
Incubate human brain microvascular endothelial cells with medium of no EGT (control group) and medium of EGT (0.025%). Then perform Western blot analysis. The amount of protein is normalized with B-Actin. Values are the average of three independent experiments.
*Compared to the control group, P, 0.05.

J Pharmacol Exp Ther 350:691–700

Efficacy of Ergothioneine--Super Synergistic Effect



EGT works synergistically with VC to protect the stability of VC (extracellular)

Experiment:

1. EGT mixed with 2 mM Ascorbic acid in ratios: 1:10 ~ 1:500.
2. Heated 37 C for 5 hrs.
3. HPLC assay for Ascorbic Acid.

Conclusion:

When EGT: Vc=1:10 is the most efficient, the remaining Vc content can be increased by 1,070%.

WO 2019/173159 A8

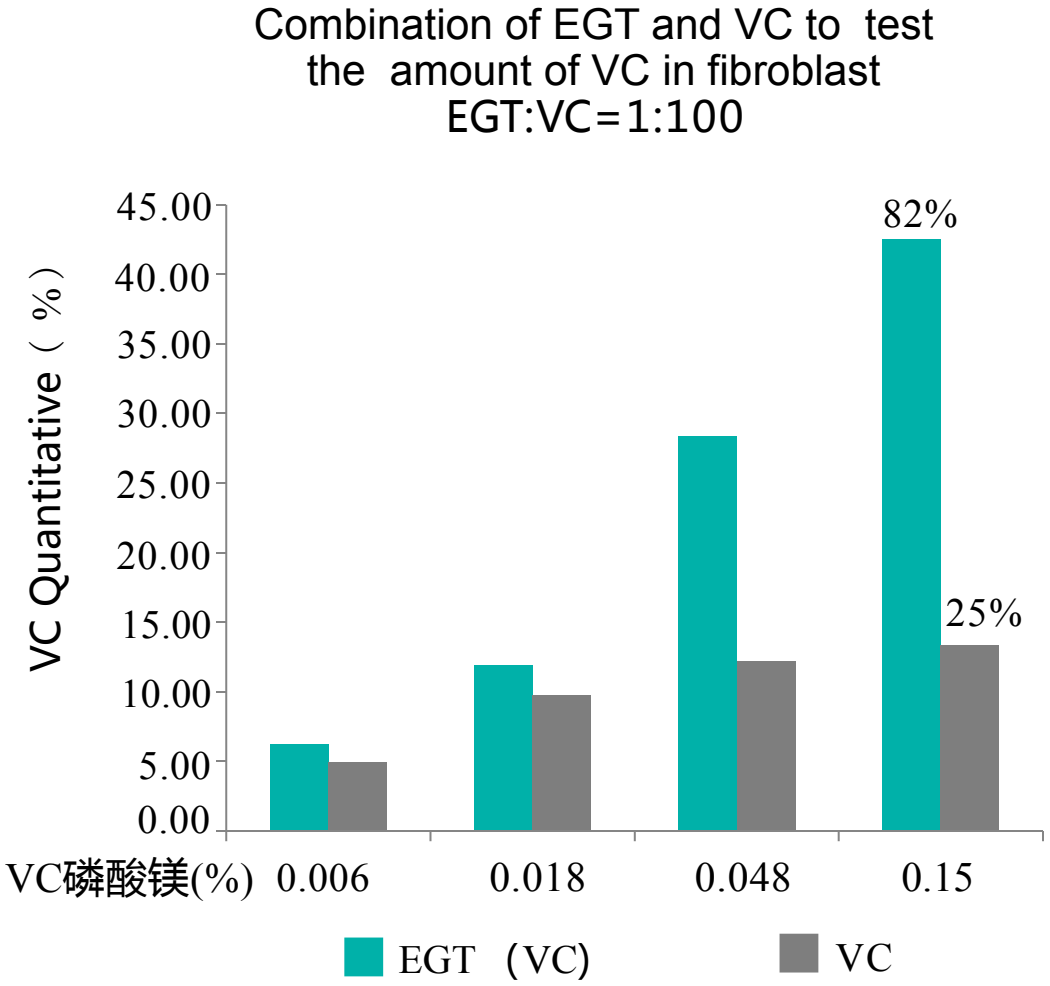
Efficacy of Ergothioneine--Super Synergistic Effect

EGT works synergistically with VC to protect the stability of VC and to enhance the activity of VC (intracellular)

Experiment:
Adult fibroblasts were cultured, an equal amount of VC derivatives were added, EGT was added in a certain proportion. With certain treatment, a large number of free radicals were released to destroy VC, and after 18 hours of incubation, the remaining content of VC in the cells was collected and measured.

Conclusion:
When EGT: VC magnesium phosphate = 1:100, the remaining VC content can be increased by 300%.

EGT not only act as a strong antioxidant, but also enhances the activity of VC. EGT has a good synergistic effect, and can achieve the effect of 1+1>2.

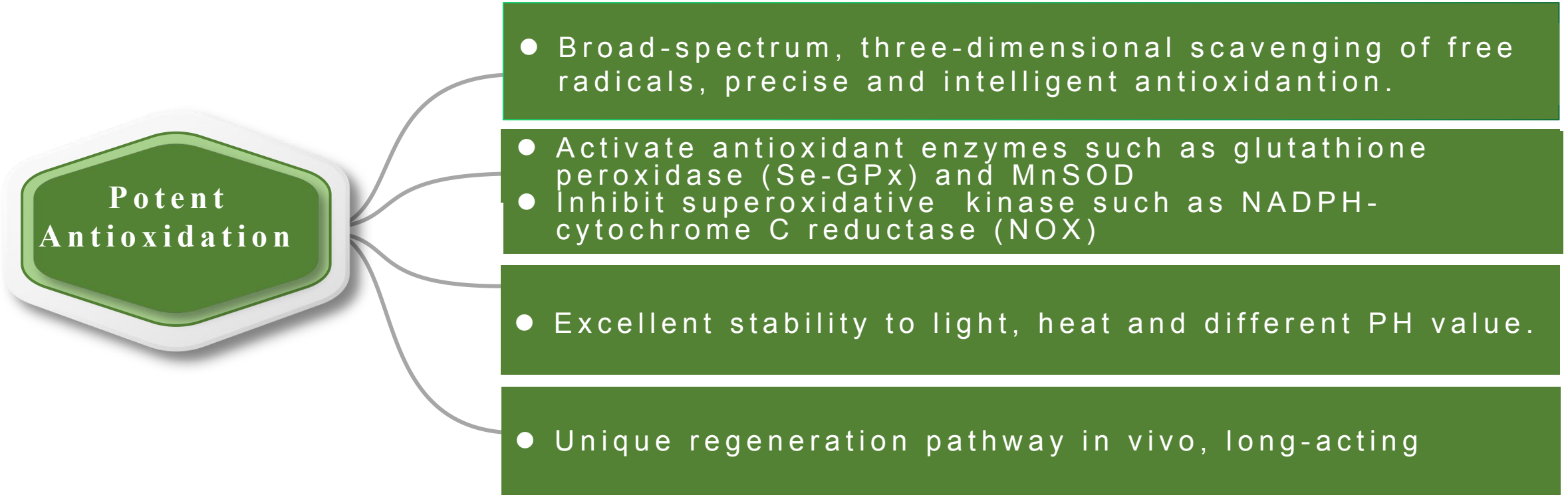


WO 2019/173159 A8/Biochem J 315: 625 –629, 1996.

Efficacy of Ergothioneine--Multi-functional PhysiologicalCytoprotector

ANTI-AGING

- EGT is an important active substance in our human body. It is recognized as a unique, multi-functional physiological cytoprotector. It has the functions of scavenging free radicals, detoxification, maintaining DNA stability, cell reproduction, cellular immunity, anti-radiation, whitening and anti-aging, anti-glycation and antioxidantion.



Efficacy of Ergothioneine--Downregulate the Gene Expression Level of MMP-1

ANTI-AGING

EGT downregulates the gene expression level of MMP-1

Mechanism:

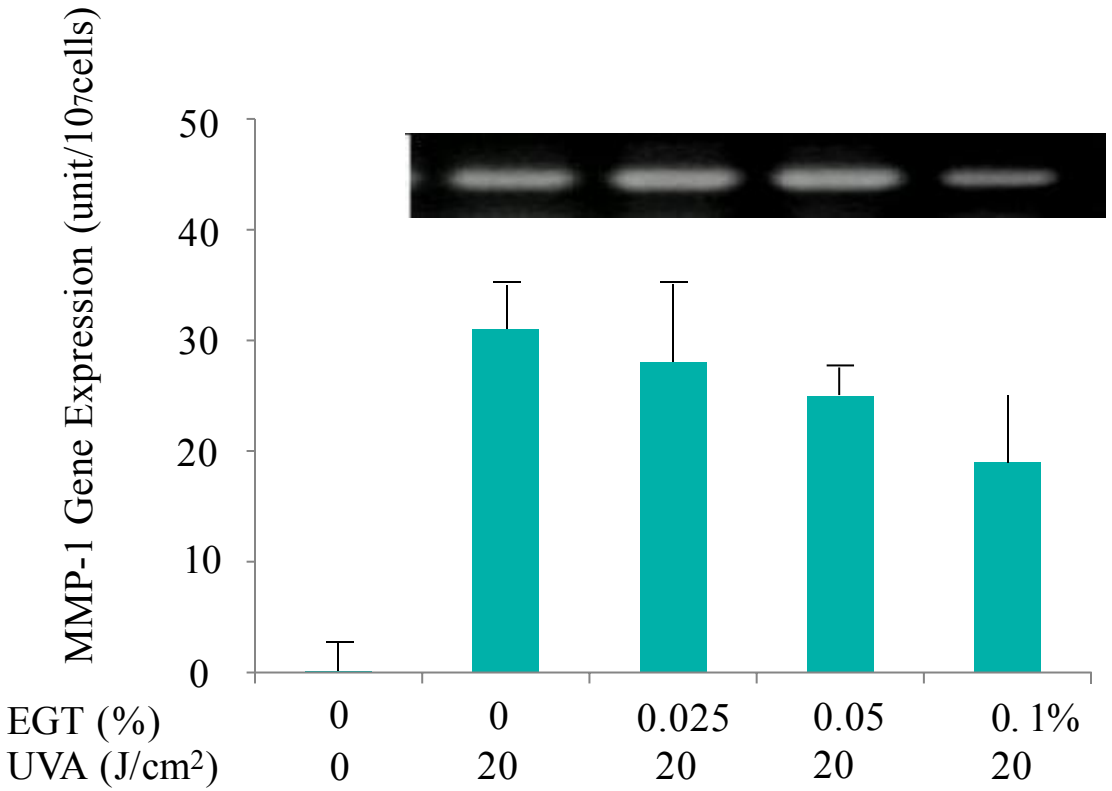
UVA irradiation to the skin causes increased expression of the gene MMP-1, thereby destroys human collagen and eventually forms photoaging of the skin.

Experiment:

Use different concentration of EGT to culture human fibroblasts. UVA (20 J/cm2) irradiated, and after 24 hours of incubation, cells were collected to measure MMP-1 activity in cells.

Conclusion:

With a concentration of 0.1% EGT, the inhibition rate of MMP-1 activity reached 56%.



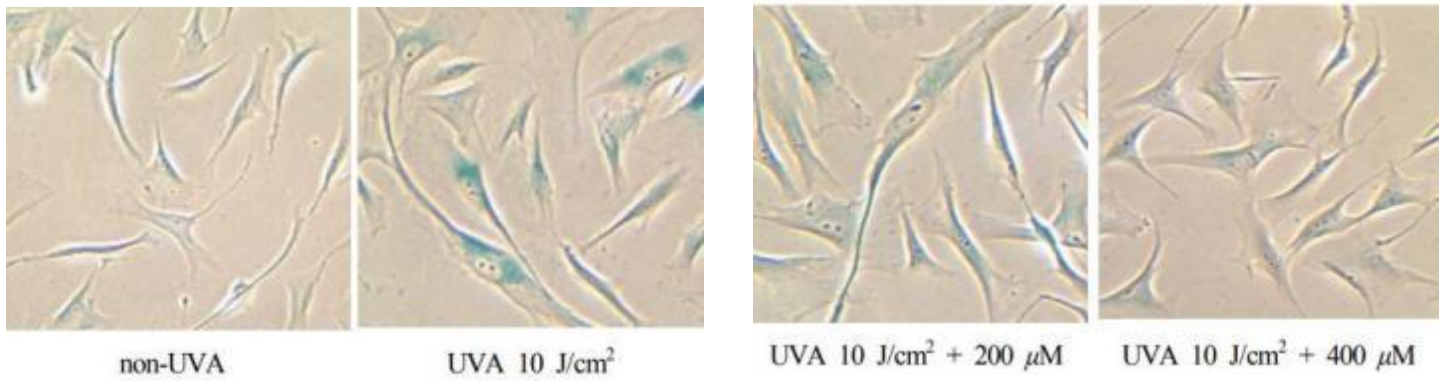
Obayashi et al., J. Cosmet. Sci. 56:17, 2005

Efficacy of Ergothioneine--Inhibit Glycation

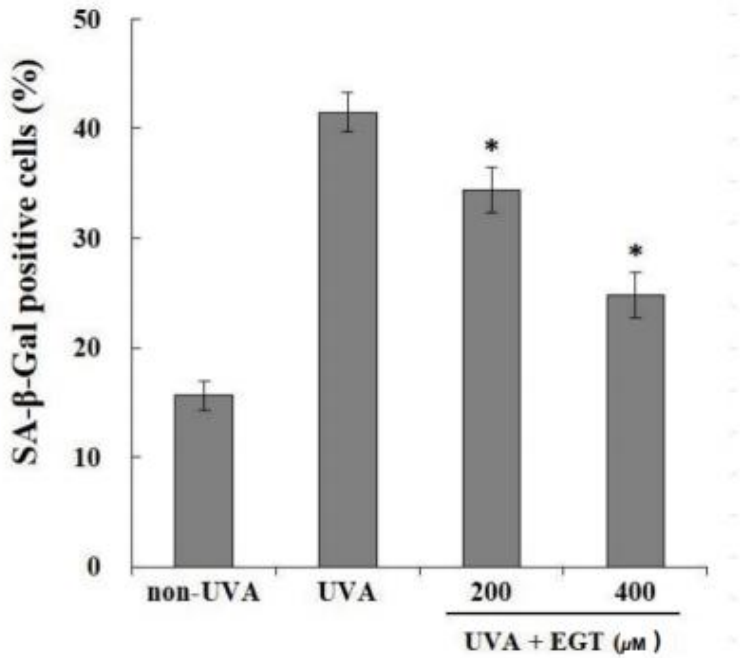
ANTI-AGING

Experiment of inhibiting glycation

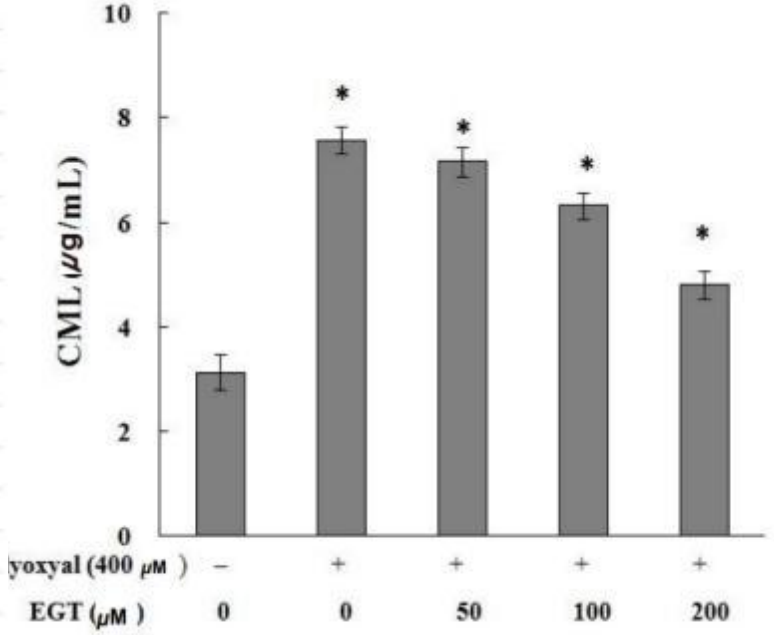
- Pretreate the cells with concentration of 0.01% EGT, the activity of SA-β-gal (β-galactosidase) is inhibited by 42%.
- Pretreate the cells with concentration of 0.005% EGT, Glyoxdehyde-induced N ε-CML is reduced by 35%.



SA-β-gal (β galactosidase) cell experiment



- Pretreatment of cells with 0.01% EGT could reduce SA-β-gal (β galactosidase) in cells by 42%.



- 0.005% EGT could reduce glyoxal-induced N ε-CML by 35%.

J. Soc. Cosmet. Sci. Korea, Vol. 45, No. 2, 2019

Efficacy of Ergothioneine--Protect Elastin

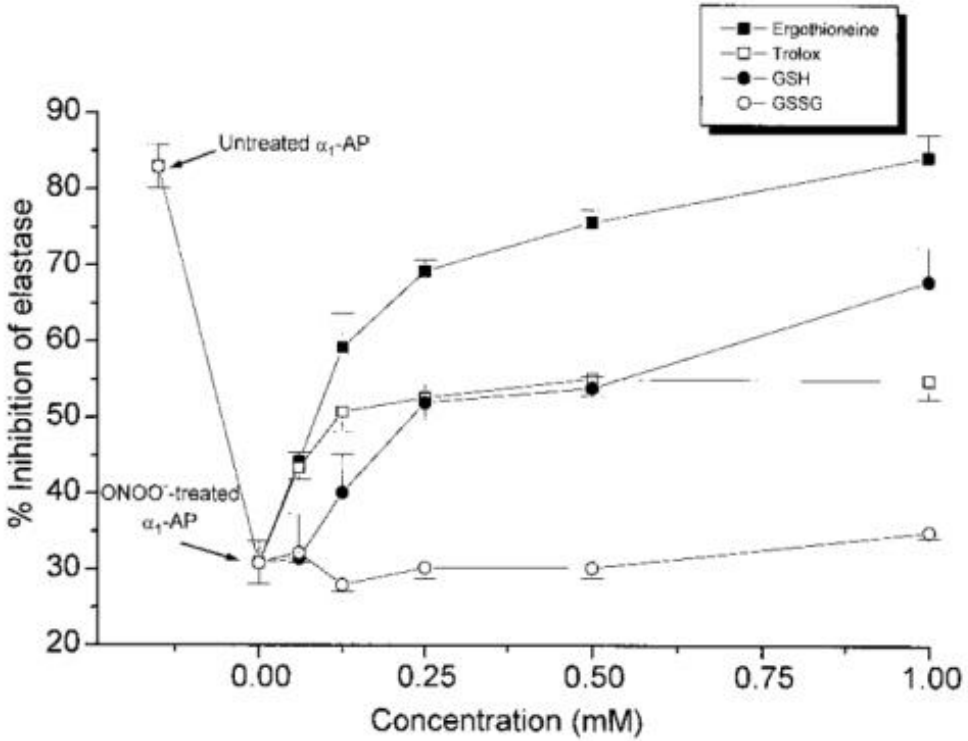
Experiment of EGT protecting elastin

Mechanism:

- α1-antitrypsin is a glycoprotein. Its function is to inhibit trypsin, chymotrypsin, hyaluronidase, plasmin, elastase, etc.
- EGT could protect α1-antitrypsin so as to inhibit elastase, and protects elastin.

Conclusion:

With 0.025% EGT, the protection rate of elastin reaches 83%.

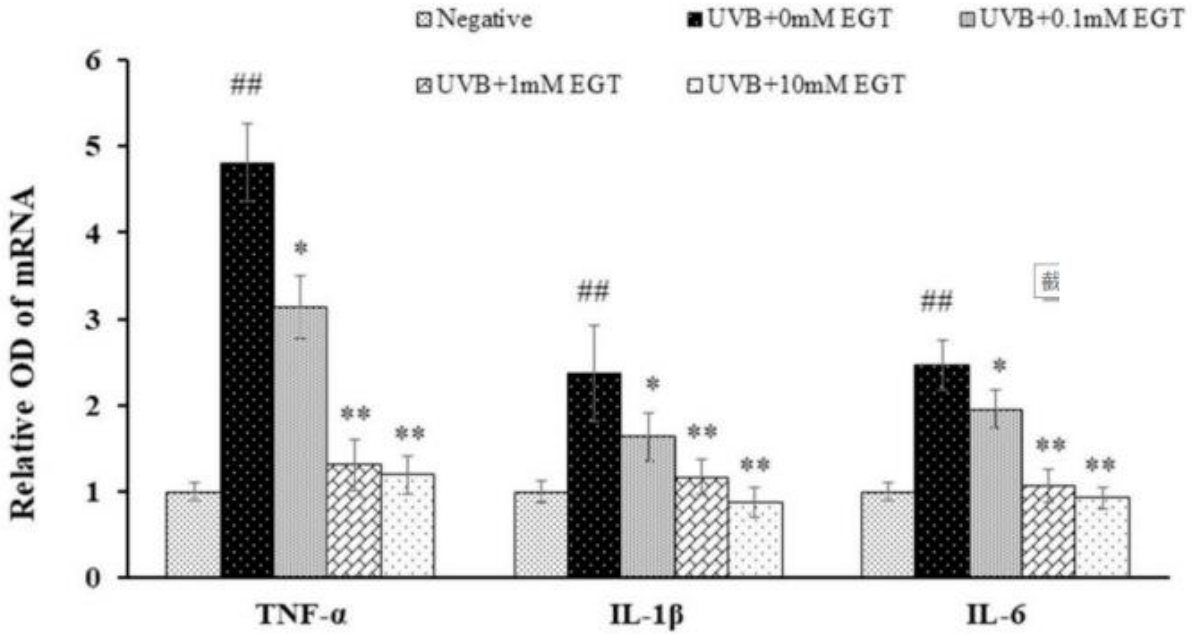


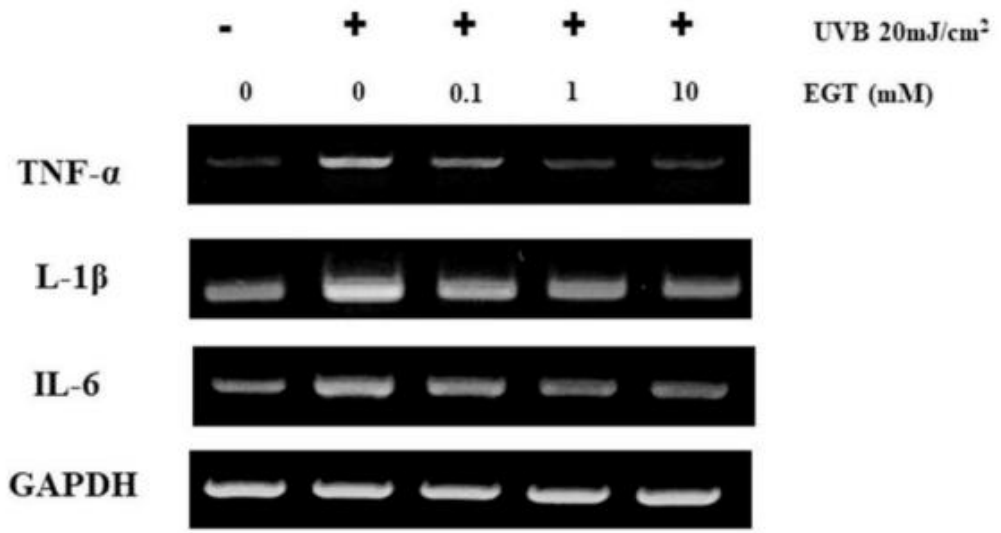
Biochem Biophys Res Commun. 1997 Feb 13;231(2):389-91.

Efficacy of Ergothioneine--Inhibit the Production of Multiple Proinflammatory Cytokines

ANTI-AGING

EGT inhibits the production of multiple proinflammatory cytokines in UVB-induced HaCaT cells





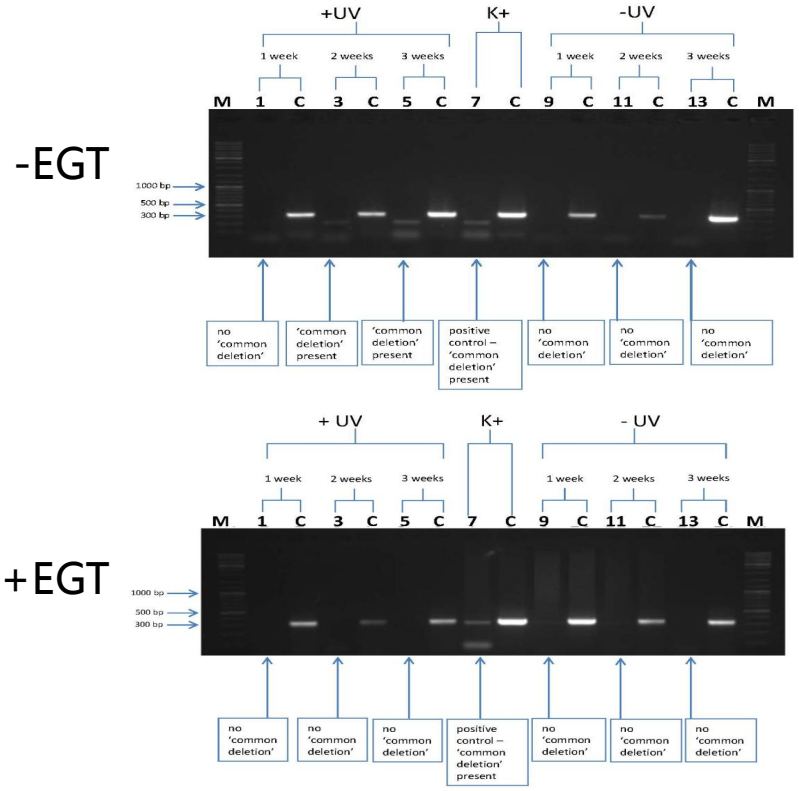
- Treat keratinocytes with different concentrations of EGT, irradiate keratinocytes with UVB, and then detect the mRNA levels of proinflammatory cytokines (TNF-α, IL-1β, and IL-6) by RT-PCR.
- GAPDH is used as an internal control. Quantitative normalization of proinflammatory cytokines is the the expression after GAPDH.
- #$p < 0.05$ versus negative control; *$p < 0.05$, **$p < 0.01$ versus vector-treated HaCaT cells.

Experimental Cell Research 400 (2021) 112516 Obayashi et al., J. Cosmet. Sci. 56:17, 2005

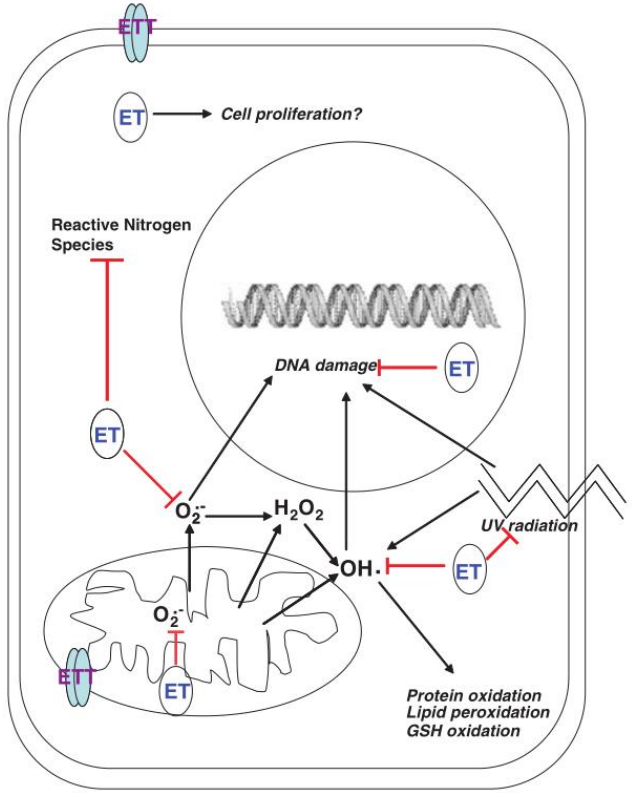
Sun protection and UV resistance functions of EGT

EGT has powerful antioxidant properties and can absorb part of UV D, which can increase the brightening effect. For example, EGT has a strong ability to clear OH. and protects cells from UV-induced ROS damage. At the same time, EGT can also activate the gene of auto-antioxidant (NrF2), improving the body's ability to resist oxidative damage to the skin caused by ROS.

- Representative PCR analysis of "common deletions" in human fibroblasts



- Potential molecular mechanism of EGT resistance to ultraviolet

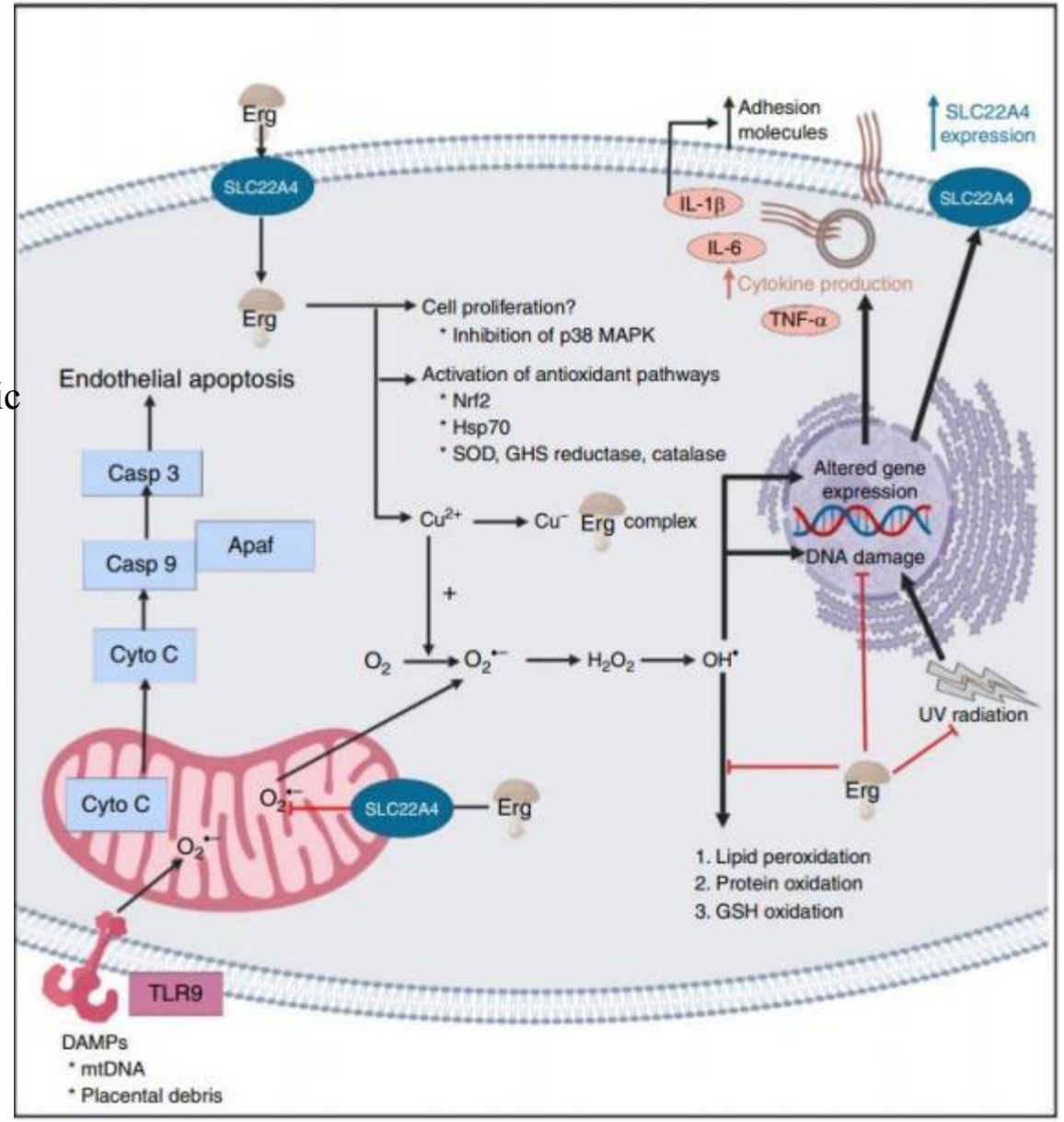


Efficacy of Ergothioneine--Nine features

ANTI-AGING

Long-acting natural antioxidant, target intelligently in mitochondria and nucleus

1. Natural: naturally existing amino acid.
2. Stable: stable to light, high temperature, and also different pH.
3. Non-allergic: suitable for all kinds of formulas without causing allergic reaction to all skin.
4. Super strong: super antioxidant capacity.
5. Broad-spectrum: scavenge free radicals in a broad-spectrum way.
6. Targeting: has exclusive tranporter OCTN-1 to carry EGT into mitochondria and nucleus of inflammatory and damaged cells.
7. Durable: half-life is 730 hours in vivo, which is 200 times of Vc.
8. Balanced: only remove excessive ROS that damage the living body, and do not affect the function of normal free radicals
9. Synergistic: able to stabilize Vc and derivatives, astaxanthin, photoglycyrrhizine, retinol and derivatives.



Nutr Res RevNutr Res Rev. 2020 Dec;33(2):190 -217.

Efficacy of Ergothioneine--Recommendation Dose

Efficacy Claim	Volume of Addition
Synergy with actives	0.0001%-0.001%
Antioxidant effect	0.01%-0.1%
Whitening	0.02%
Whitening, brightening, anti-inflammatory, repairing	0.05%
Anti-aging, sensitive skin repair	0.10%
Rapid anti-aging, removes fat particles	0.50%
Sun protection, anti-photoaging	2.00%
Oral dosage	30mg/adult/day, 20mg/child/day,
Formula： PH<7	Water, lotions, creams, essences, masks

麦角硫因
延 缓 衰 老

03

Company Introduction

- Shanghai EGT Synbio Group Co., Ltd. is committed to the mission of delaying aging. Relying on two major technology platforms of **biological fermentation** and **enzyme-directional evolution**, we could provide excellence and effective natural anti-aging active ingredients for food, health care, cosmetics and pharmaceutical industries.
- With modern biotechnology, we have realized the world's first large-scale biological process of producing 100% L-(+)-ergothioneine. The purity of our ergothioneine is up to 99%, and the optical rotation is ≧+122° . Our ergothioneine is white crystal, odorless, and non-hygroscopic, and has no residues of "toxic solvent" and "D-ergothioneine".

Company Introduction--Different Production Processes

Chemical Synthesis

- The synthesis is difficult, and the expected yield cannot be achieved due to partial or total racemization.
- The safety is difficult to be guaranteed, the synthetic raw materials are expensive, and the synthetic cost is high

Natural Extraction

- Extracted from the fruiting bodies of edible fungi, ergots and grains, but with low content.
- There are many impurities in the raw materials, pesticide residues, and the extraction cost is high.

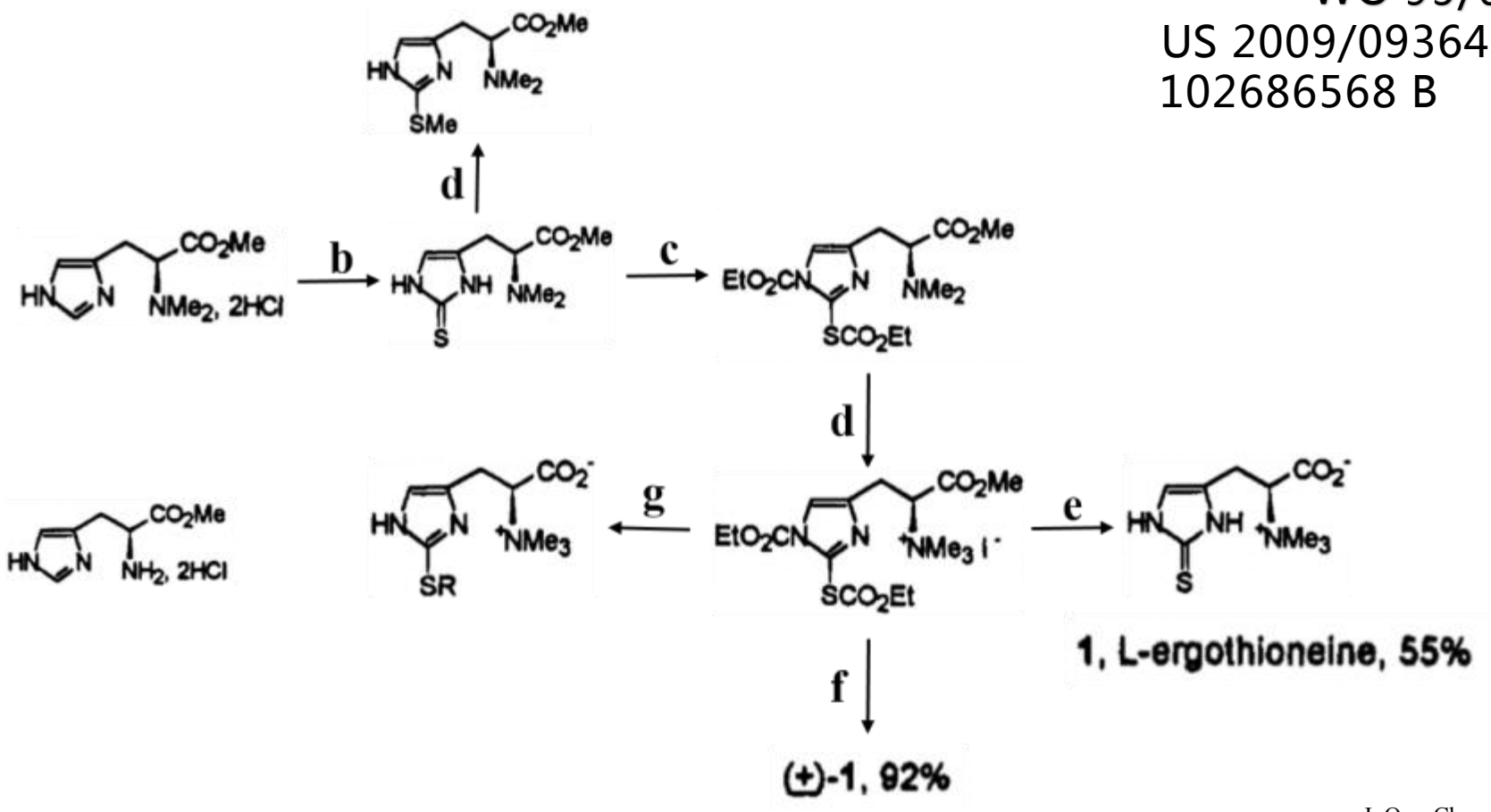
Biosynthesis

- Biotechnology, with good safety, is one of the mainstream directions of low-cost large-scale production;
- Current Problems: the fermentation efficiency is low, the purification difficulty is high, and the cost is high.

Chemical Synthesis

Oxis International, Tetrahedron et al (法国四面体公司)

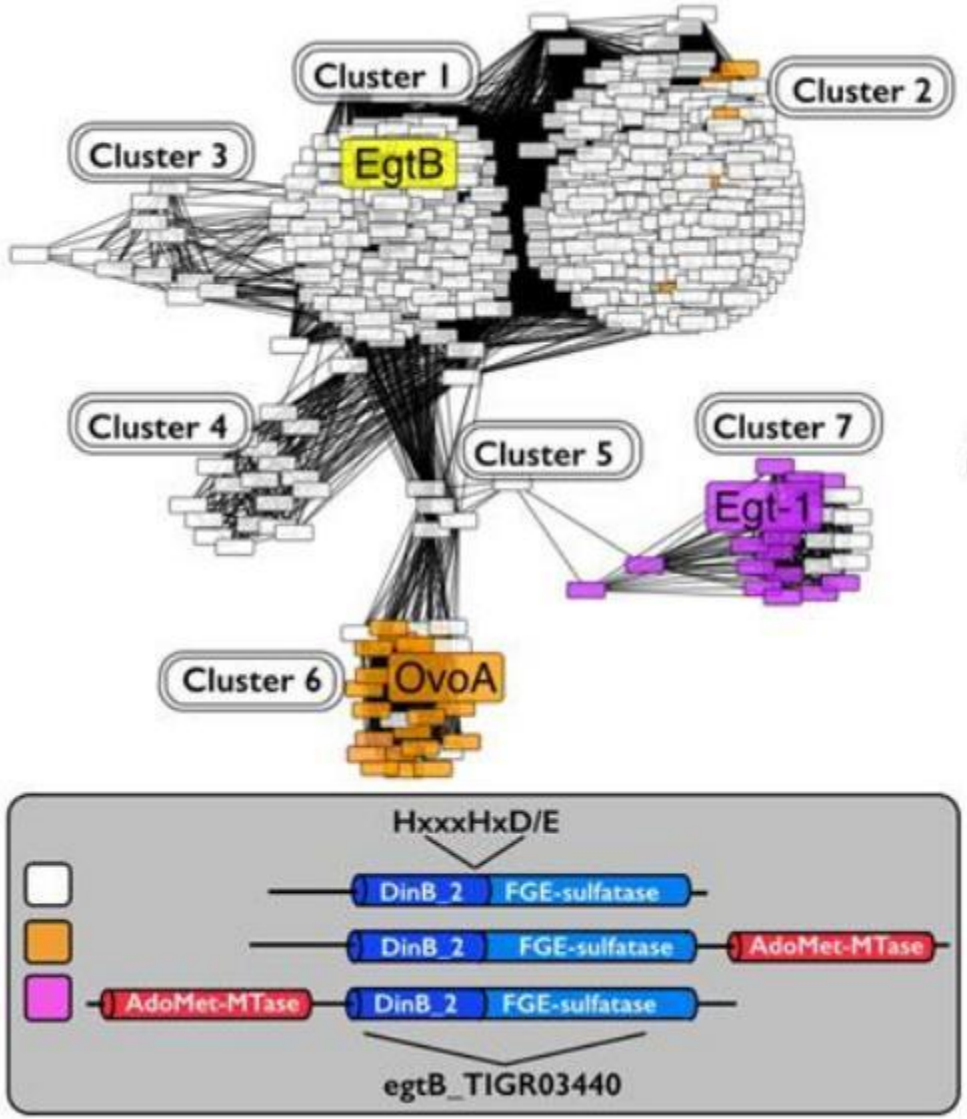
WO 95/00494
US 2009/093642 CN
102686568 B

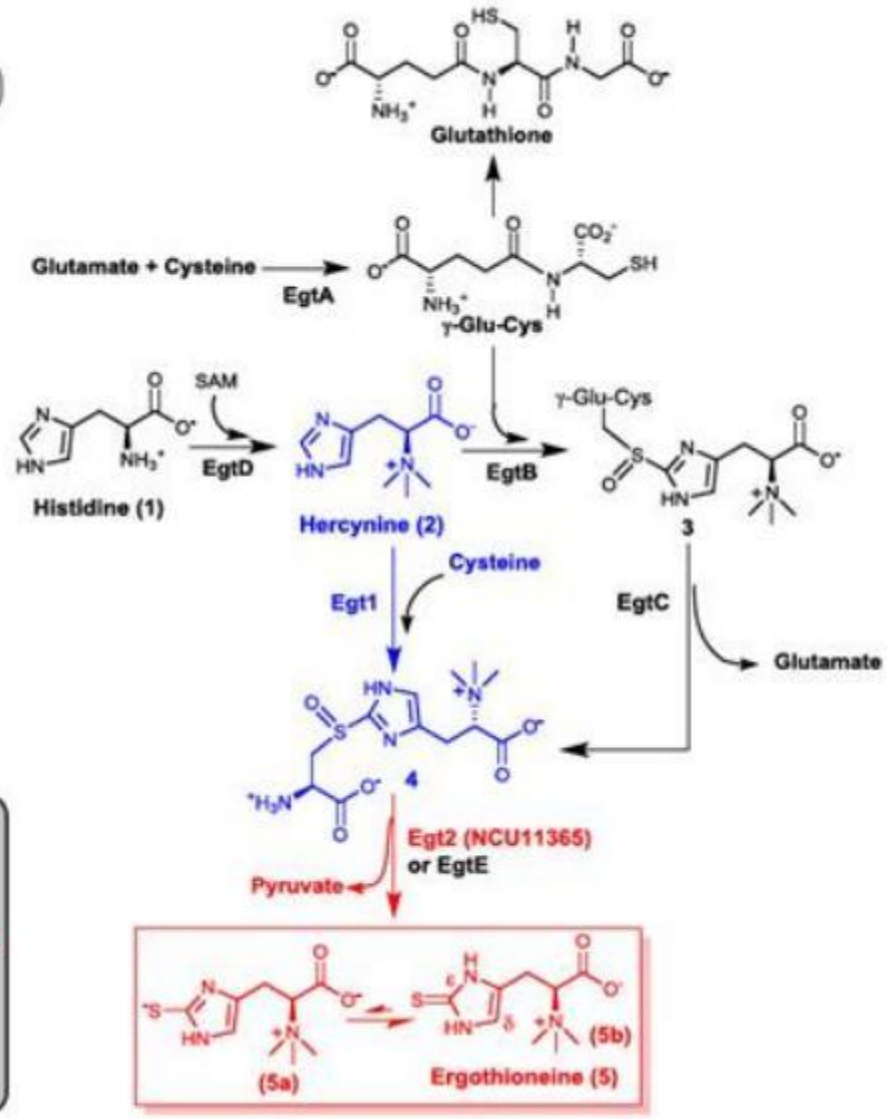


J. Org. Chem. **1995**, 60, 6296-6301

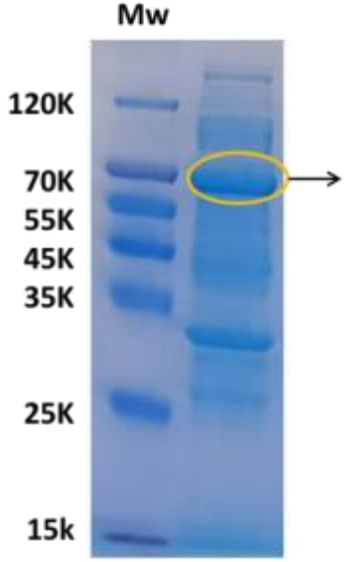
Company Introduction--We Imitate the Natural Biosynthetic Pathway of Mushrooms

ANTI-AGING

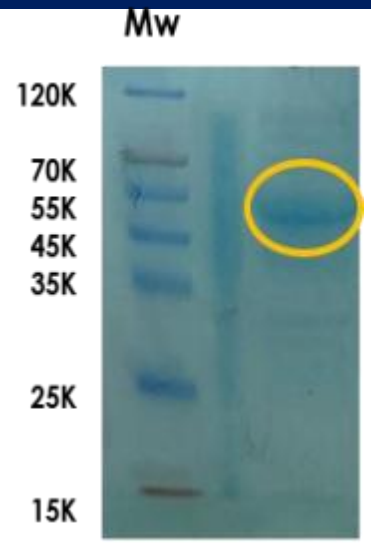




Innovative and efficient production process



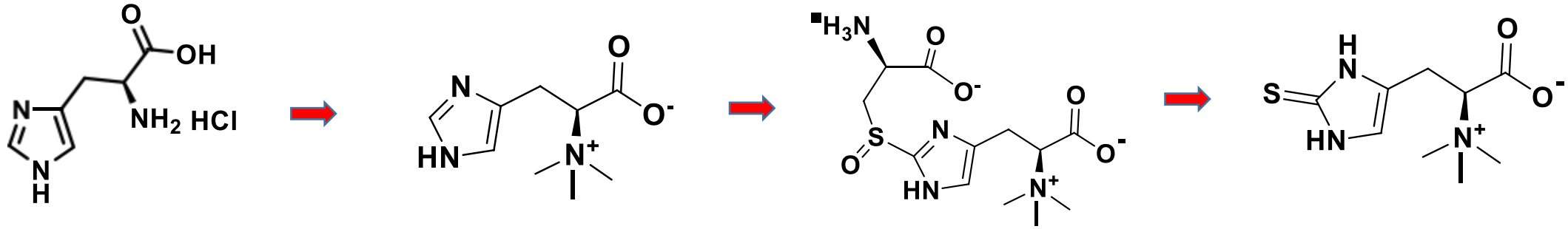
EGT1



EGT2

L- Hercynine → EGT1 catalysis (100%) → EGT2 catalysis (100%) → Purification (Crystallization) → L- Ergothioneine

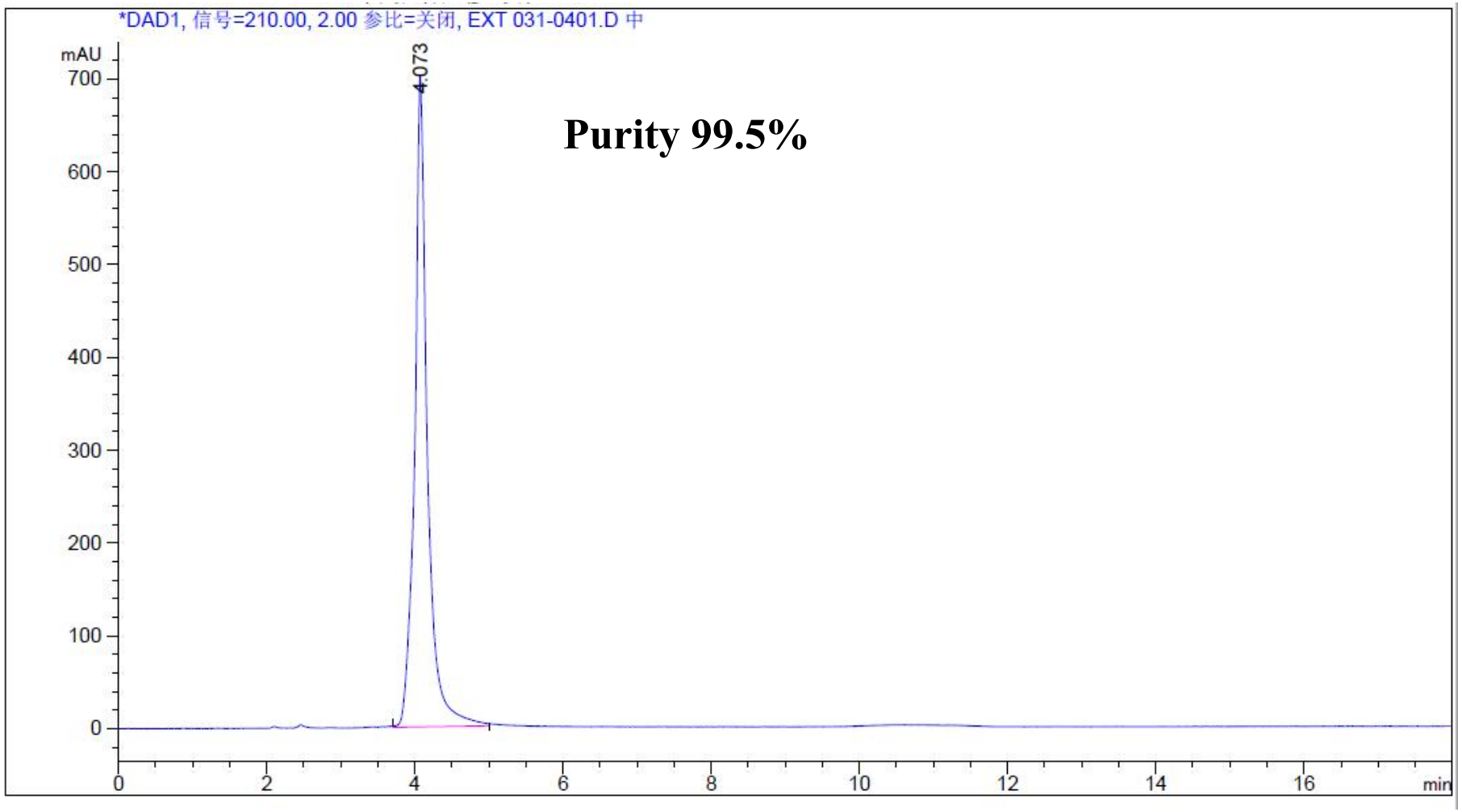
> 80% Yield
> 99% Purity



Company Introduction--HPLC Spectrogram to detect the Purity of L+Ergothioneine

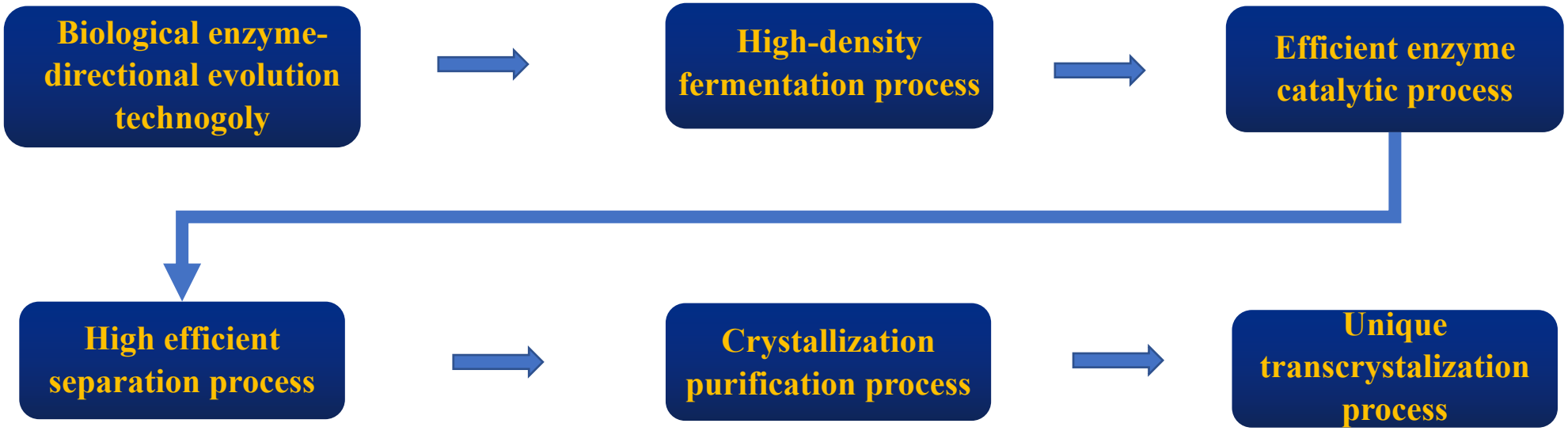
ANTI-AGING

The perfect combination of chemistry and biotechnology



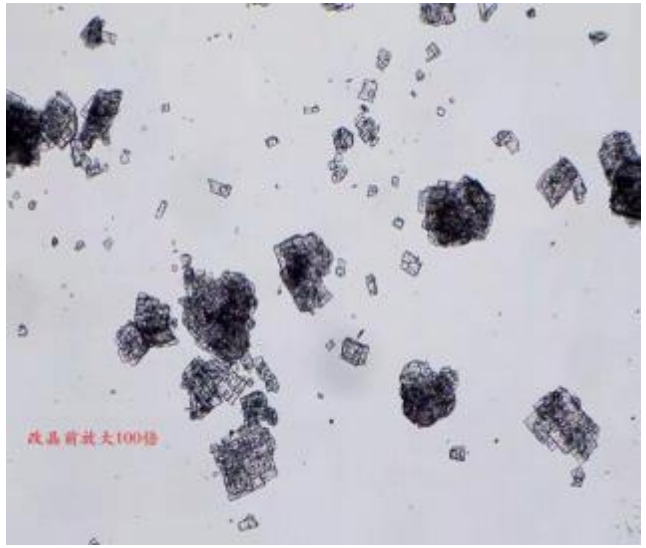
Purity 99.5%

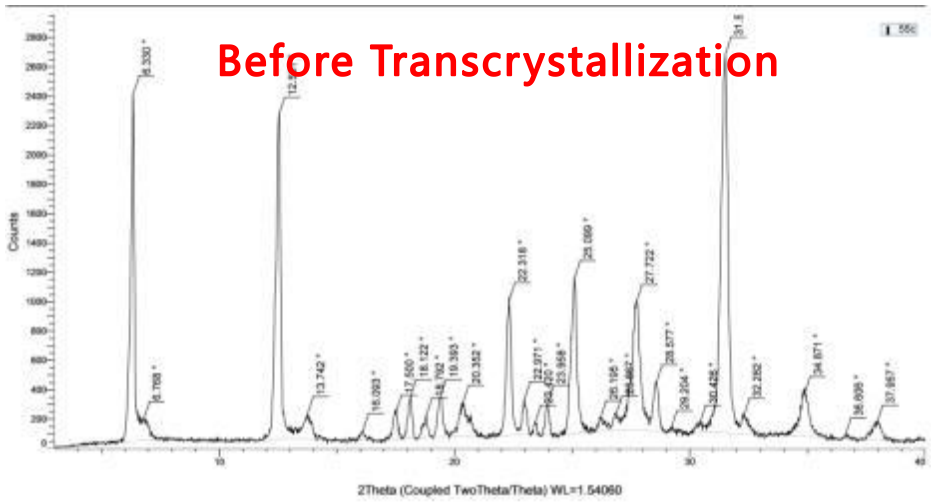
Company Introduction--The perfect combination of chemistry and biotechnology



Full chain technical solution for Ergothioneine Production

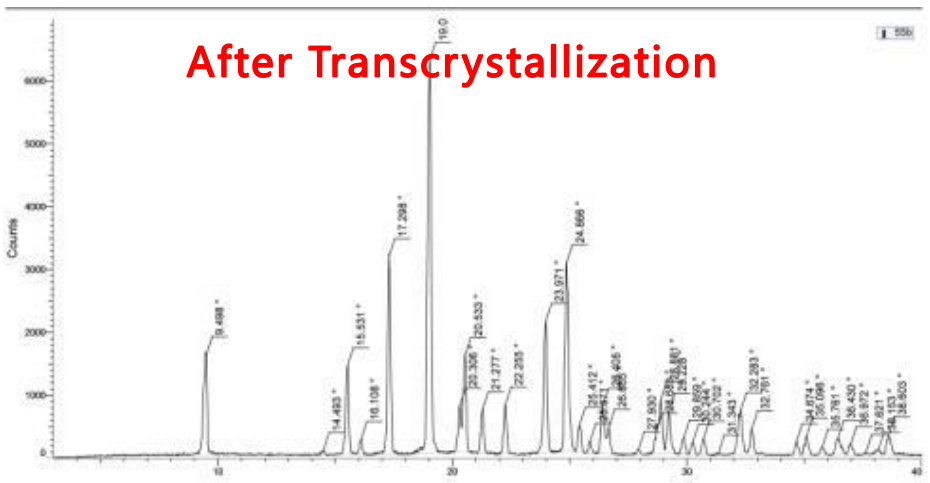
High quality assurance - unique and advanced transcrystallization technolgoy

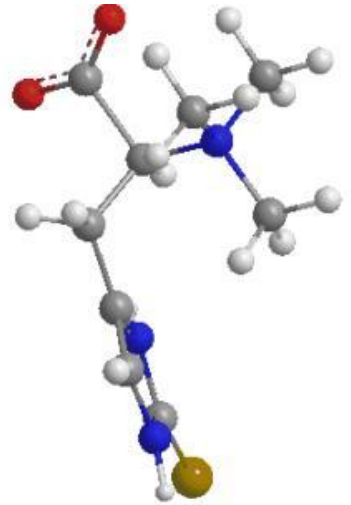




Before transcrystallization:
hygroscopic, odorous, powder

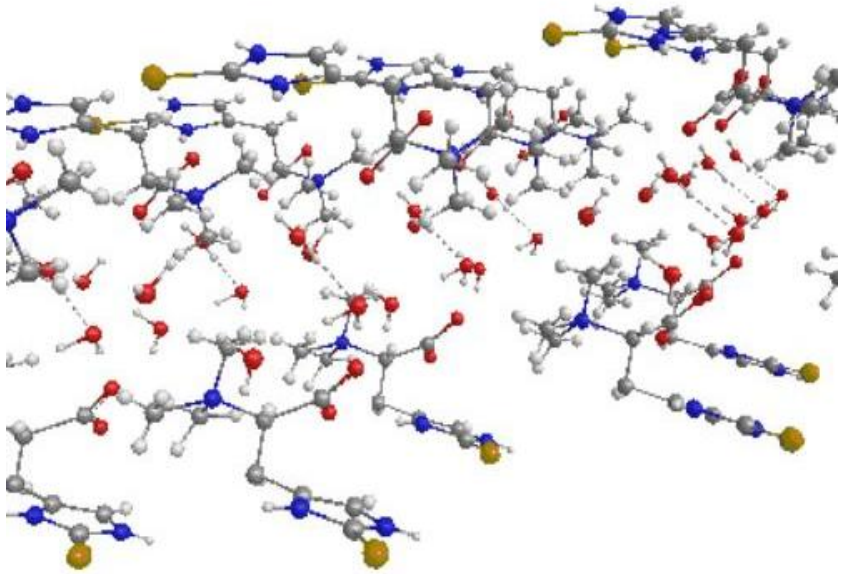
After transcrystallization:
non-hygroscopic, odorless, crystal

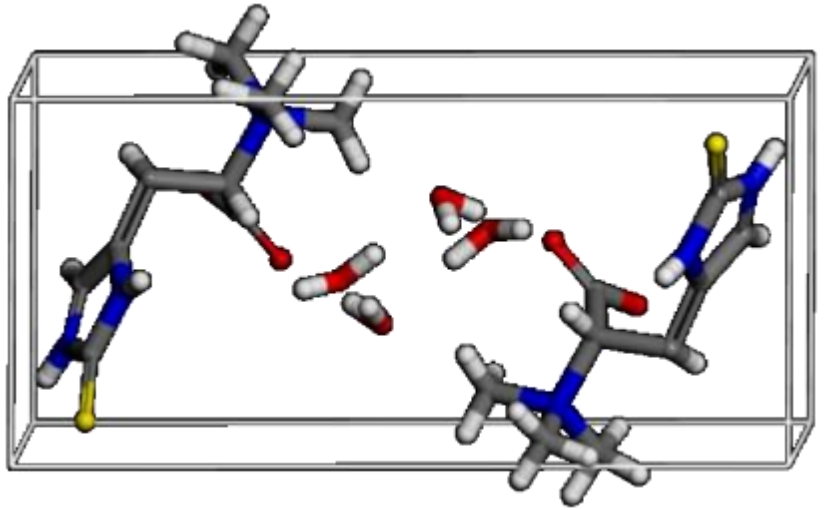




Crystal molecule structure of other company

- In this crystal structure, EGT is relative and parallel stacked. The molecular spacing is large, which is conducive to the entry of water molecules and forms a strong hydrogen bond with C=O.
- Therefore, EGT of this structure is easily hygroscopic and odorous.

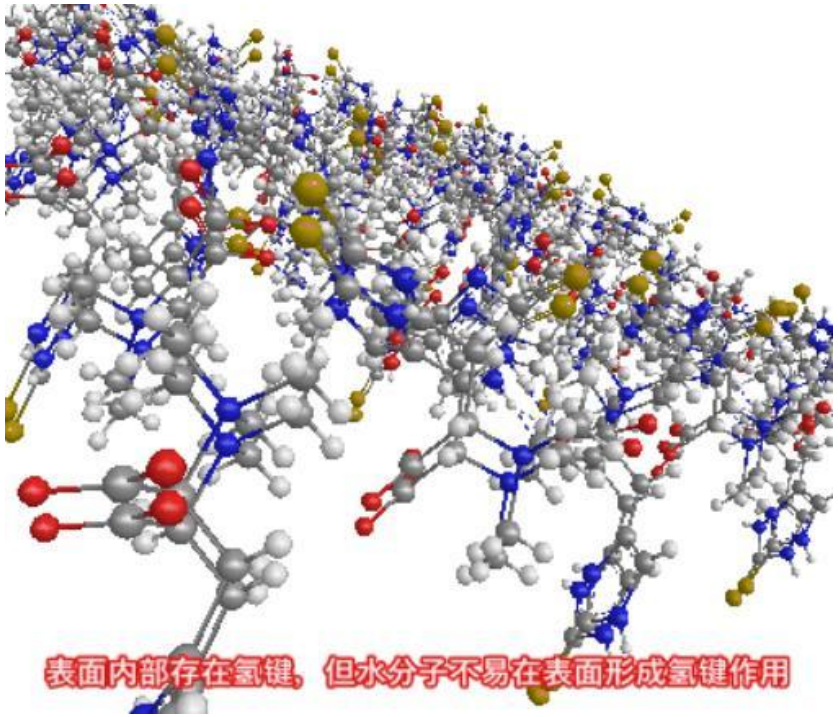


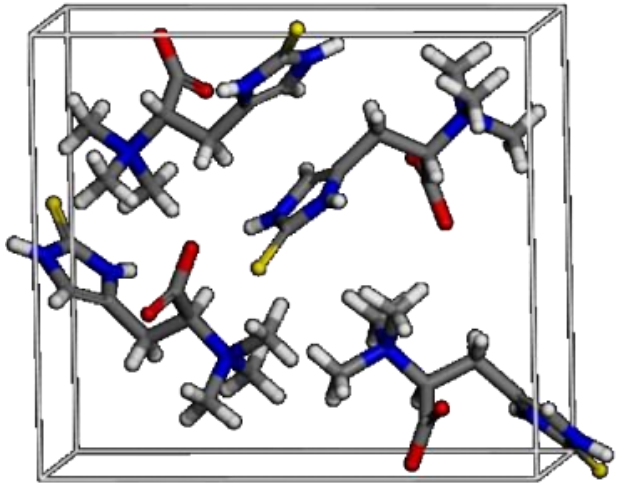


Space clusters structure:P21
a=7.171
b=6.129
c=15.051
β=90.83°

Transcrystallization molecule structure of our company

- In this crystal structure, EGT molecules are staggered and stacked in crossing way. There are a large number of hydrogen bonds on the crystal plane of the molecule, which are tightly stacked, and water molecules are not easy to form hydrogen bonds on the surface.
- Therefore, EGT of this structure is not easily hygroscopic, and also it is odorless.





Space clusters structure: P212121
a=6.1256
b=12.488
c=14.007
A=β=γ=90.00

Advanced production equipments

Advanced testing equipments

Quality Control

The raw materials and finished products of each batch are strictly tested, and the fermentation technology is tracked throughout the process.

With advanced production technology and strict quality control, we can product several tons of EGT every year. Our EGT is featured the best quality and the lowest prict.

SPECIFICATION OF ERGOTHIONEINE

Analysis Item	Specification	Test Method
Appearance	White crystal	Visual inspection
Melting point	275-277℃	Melting point apparatus
Purity	≥99.5%	HPLC (Normalization method)
Optical rotation	$[\alpha]_D \geq (+)122°$	Polarimeter (c=1, H_2O)
Loss on drying	<0.5%	Thermogravimetry
Identification	The NMR spectrum is consistent with the standard spectrum.	NMR
Elemental analysis	C: 47.14±0.3%, H: 6.59±0.3%, N: 18.32±0.3%,	Elemental analysis
Residual solvent (Alcohol)	< 1000 ppm	GC

Company Introduction--Advantages of Our EGT

01 New process

- Imitate the biosynthetic pathway of ergothioneine and use advanced synthetic biology techniques.

02 High purity

- The purity of our ergothiogen is as high as 99.5%, white crystal, non-hygroscopic and odorless.

03 Good price

- The market price has been less than one-third of the original. As the market expands, we will continue to reduce the price in the future.

Company Introduction--Our company and Culture

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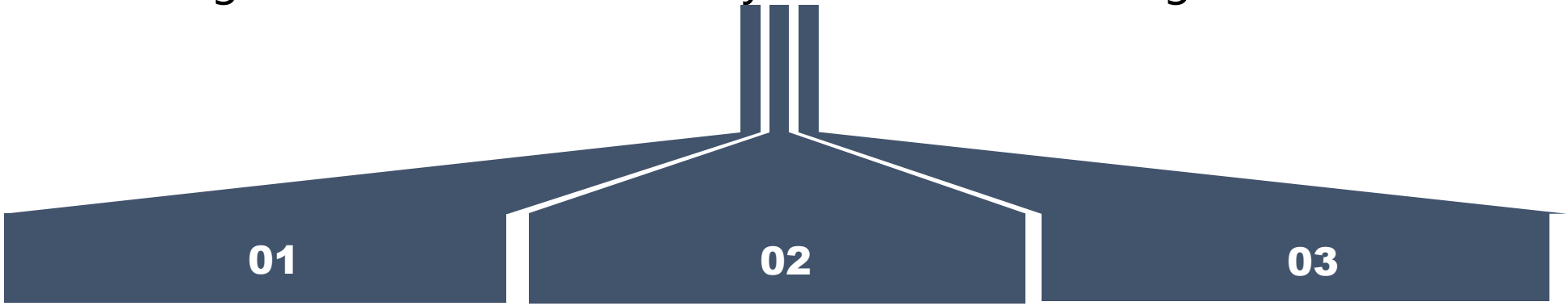
Shanghai EGT Synbio Group Co., Ltd. is committed to the mission of delaying aging. Relying on two major technology platforms of biological fermentation and enzyme-directional evolution, we keep on continuous improvement and provide natural anti-aging active ingredients for food, health care, cosmetics and pharmaceutical industries. Its subsidiary, YG Ingredirnts, was established in 2016. We have more than ten R&D talents and a scientific advisory committee chaired by researchers from the Chinese Academy of Sciences. Our company focuses on biological fermentation and enzyme catalysis technology, after thousands of experiments, we have made continuous breakthrough in four aspects: strain screening, combined fermentation, enzyme directional evolution, and transcrystal purification. we realized the facility startup of the world's first large-scale biological process of producing ergothioneine in 2021. The purity of our ergothioneine is up to 99%, and the optical rotation is ≧+122°. Our ergothioneine is white crystal, odorless, and non-hygroscopic, and also has no residues of "toxic solvent" and "D-ergothioneine".

Our vision is to be the No.1 international supppier for Ergothioneine and Let everyone benefit from Ergothioneine.



Focus on one thing and do it well.

麦角硫因
延 缓 衰 老

Youthful Skin - Ergothioneine

Targeting intelligently mitochondria and nucleus, Long-acting, and Natural Antioxidant

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