DermaPep™ A440

Innovative Anti-Aging Tetrapeptide

TRI-K
A MEMBER OF THE Galaxy GROUP

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DermaPep
Experience the magic of science

• Anti-aging

DermaPep™ A350

DermaPep™ A420

DermaPep™ A440

DermaPep™ A530

• Whitening

DermaPep™ A350

DermaPep™ UL

DermaPep™ W220

DermaPep™ W411

• Anti-inflammatory

DermaPep™ A350

DermaPep™ A440

DermaPep™ A530

DermaPep™ UL

DermaPep™ W220

DermaPep™ W411

DermaPep™ A440
Anti-Aging Peptide

Introduction

Collagen represents the main component of the extracellular matrix of the dermal connective tissue and its concentration decreases as we age. It is known that the loss of collagen, especially in photoaged skin, is not only due to a reduction of the synthesis but also due to its enhanced degradation. The enzyme mainly responsible for collagen breakdown and inhibition of new collagen formation in the skin is MMP-1 which cleaves collagen type I, III, VII, VIII. Exposure of the skin to UV radiation results in the upregulation of several different MMPs and the repeated upregulation of these collagen-degrading enzymes over time is thought to underlie the collagen damage that is one of the hallmarks of photoaging.

In a healthy skin, the synthesis and degradation of the matrix are well in balance. However, this intricate balance gets disrupted as we age, in which too little of the matrix is synthesized and too much is degraded. Many of well-known skin aging treatments are aimed at replenishing skin matrix by stimulating the synthesis of the matrix. Unfortunately, this approach fails in some people, especially old people, whose ability to respond to matrix synthesis boosters is considerably declined.

DermaPep™ A440, innovative antiaging tetrapeptide, has been developed to maximally and effectively inhibit these matrix degrading MMPs and to restore collagen-levels back to normal. In vitro results show that DermaPep™ A440 greatly inhibited the expression and production of MMP-1 under both UV-stimulated and non-stimulated condition and restored collagen levels significantly. In addition, DermaPep™ A440 considerably lowered the MMP levels that have been produced by ROS or RNS. Finally, in vivo clinical study results prove that DermaPep™ A440 is surely one of the best antiaging agents in the market.

Function

- Anti-wrinkle effect for face and body
- Reduction of MMP1 synthesis
- Restoration of procollagen type 1
- Providing exceptional anti-aging benefits
- Anti-aging effect against environmental stimulus such as UV irradiation

Applications

DermaPep™ A440 can be incorporated in cosmetic formulations such as emulsions, oily sera, gels and creams for anti-aging and anti-wrinkle purpose.

Formulation

- Dermatological tolerance : Standard testing has been performed on DermaPep™ A440 which has showed neither cytotoxic effects nor any irritation or sensitization reaction in healthy volunteers with an occlusive single patch test.
- Recommended concentration : 1-3 %

Product Information

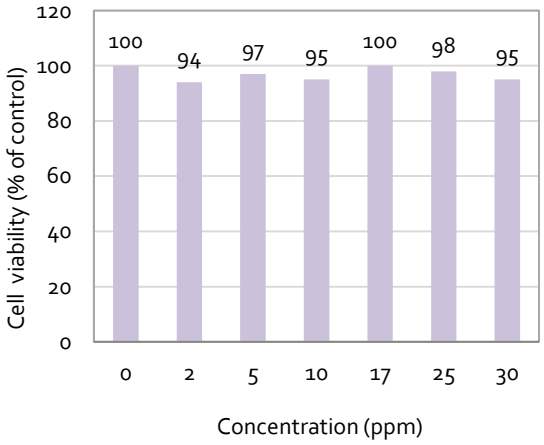
- Appearance : Transparent solution
- INCI/CTFA-Declaration : Myristoyl Tetrapeptide-34 (and) Butylene Glycol
- Active ingredient content : 0.15% Myristoyl Tetrapeptide-34
- Powder purity : 95 % up
- Preservative : None

DermaPep™ A440
Safety

Cell Cytotoxicity Test

Hs68 cells were seeded into 96-well plates (2×10^3) and treated with different concentrations of Myristoyl Tetrapeptide-34 for 24 hours. Cell viability was assessed by MTT assay and was shown relative to untreated control. Absorbance was measured by an ELISA reader at 540 nm.

MTT Assay



Skin Irritation Test

Skin irritation test was performed on 31 healthy Asian volunteers (Spincontrol Asia (study report number IR-6Q01-MW-DE08), Bangkok, Thailand).

Evaluation and grading of skin irritation

- Erythema (no redness = 0 to very strong redness=5)
  After 24 hrs, mean score = 0.00 (max = 0)
  After 48 hrs, mean score = 0.00 (max = 0)
- Oedema (no oedema = 0 to very strong oedema =5)
  After 24 hrs, mean score = 0.00 (max = 0)
  After 48 hrs, mean score = 0.00 (max = 0)
- Scaling (no scaling = 0 to very strong scaling = 5)
  After 24 hrs, mean score = 0.00 (max = 0)
  After 48 hrs, mean score = 0.00 (max = 0)

Mutagenicity (Ames Test)

Myristoyl tetrapeptide-34 at 5 different concentrations (30,60,90,180, 360 ppm) were evaluated to predict its potential to cause mutagenicity. Five different auxotrophic mutants were used to assesse directly or in the presence of liver S9 fractions.

No mutagenicity was observed.

In vitro Ocular Irritation Test

DermaPep™ A440 at 3 different concentrations (2, 4, 8 %) and butylene glycol were evaluated with the ocular irritection assay system (CA, USA).

The ocular results demonstrated that the DermaPep™ A440 was classified as mild irritants.

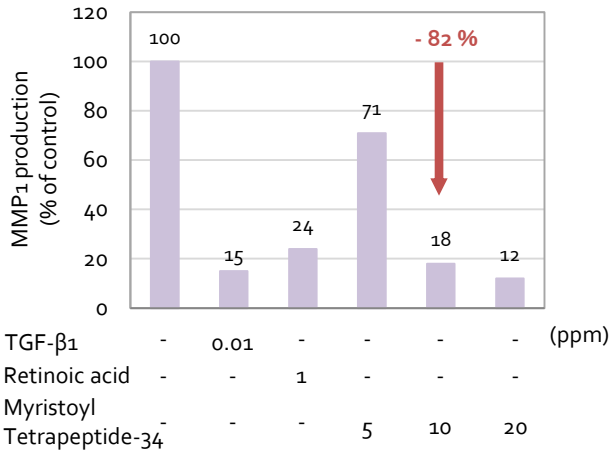
DermaPep™ A440
In vitro efficacy

Inhibition of MMP-1 Production

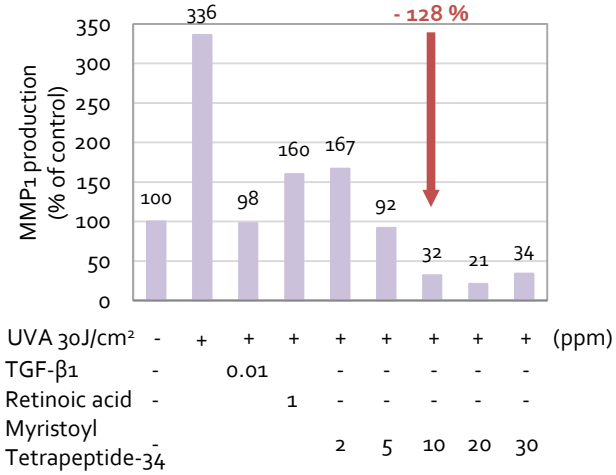
Hs68 cells were seeded into 6-well plates and treated with TGF-β, retinoic acid or Myristoyl Tetrapeptide-34 followed by UV radiation or not for 48 hours. Equal amounts of media were analyzed by ELISA assay for MMP-I. Cell viability was used for sample standardization.

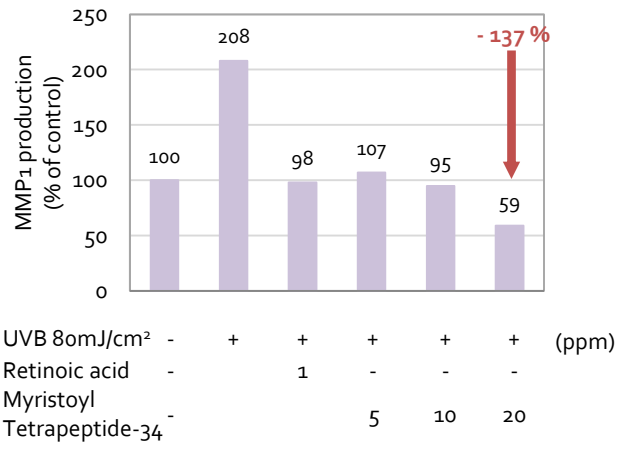
Myristoyl Tetrapeptide-34 significantly decreased MMP-1 production.

Inhibition of MMP-1 Production



Inhibition of MMP-1 production after UVA or UVB irradiation





DermaPep™ A440
In vitro efficacy

Effect on Procollagen 1 Production

Hs68 cells were seeded into 6-well plates and treated with retinoic acid or Myristoyl Tetrapeptide-34 followed by 30J/cm² UVA. After 48 hours equal amounts of cell lysates were subjected to electrophoresis and analyzed by western blot for procollagen type I.

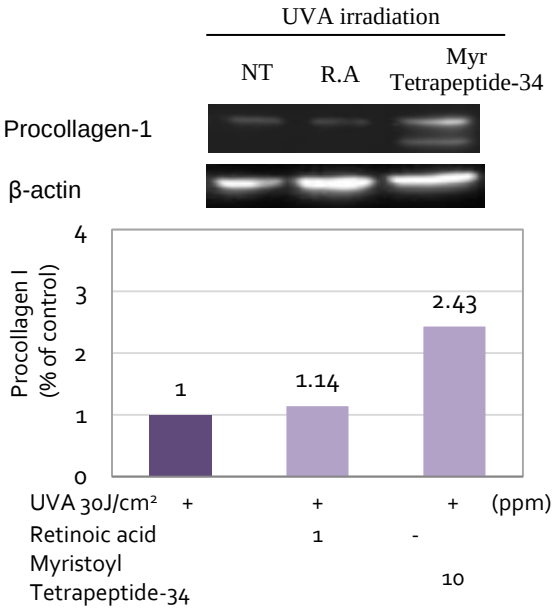
Myristoyl Tetrapeptide-34 significantly stimulated the production of type I procollagen.

Changes in MMP-1 and ECM gene expression

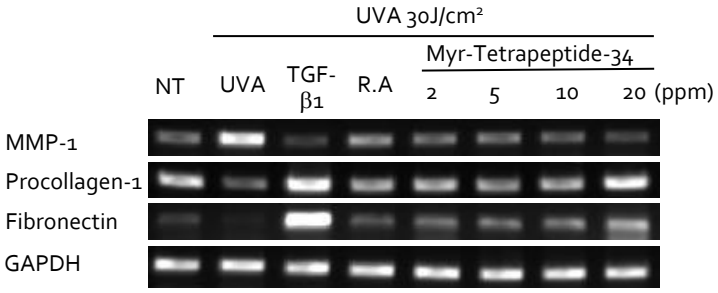
Hs68 cells were seeded into 6-well plates and treated with TGF-β, retinoic acid or Myristoyl terapeptide-34 followed by 30J/cm² UVA. After 48 hours, total cellular RNA was extracted and reverse-transcription PCR was performed to determine MMP-1, procollagen 1, fibronectin. GAPDH mRNA level was used for sample standardization.

Myristoyl Tetrapeptide-34 significantly inhibited UVA stimulated MMP-1 expression and stimulated the procollagen-1 and fibronectin expression.

Stimulation of Procollagen I Production



Changes in MMP-1 and ECM gene expression



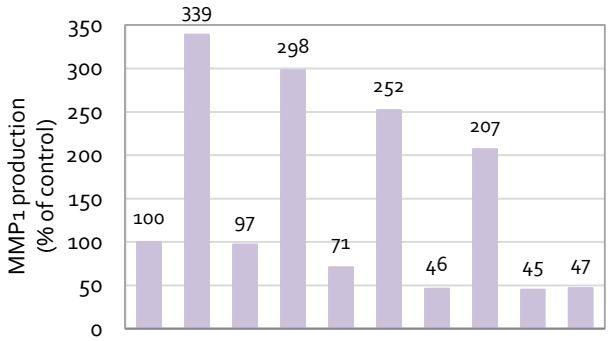
DermaPep™ A440
In vitro efficacy

Comparison with Retinoids

Hs68 cells were seeded into 6-well plates and then treated with retinyl palmitate, Myristoyl tetrapeptide-34 or both (left figure) or retinol, Myristoyl Tetrapeptide-34 or both(right figure) followed by 30J/cm² UVA . Equal amounts of media were analyzed by ELISA assay for MMP-I. Cell viability was used for sample standardization

Myristoyl Tetrapeptide-34 showed greater effect on reducing MMP-1 than retinyl palmitate or retinol alone. In addition, there was no synergy between retinyl palmitate and Myristoyl Tetrapeptide-34 or retinol and Myristoyl Tetrapeptide-34, respectively.

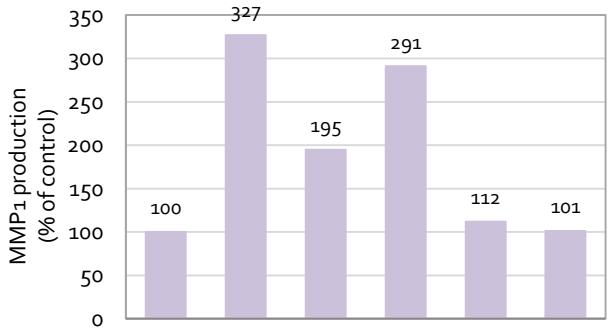
Comparison with Retinyl Palmitate on MMP-1 Inhibition



UVA 30J/cm²	-	+	+	+	+	+	+	+	+	+
Retinoic acid			1							
Retinyl palmitate				10	10	20	20	25	25	-
Myristoyl Tetrapeptide-34			-	-	10	-	10	-	10	10

(ppm)

Comparison with Retinol on MMP-1 Inhibition



UVA 30J/cm²	-	+	+	+	+	+
Retinoic acid			1	-	-	-
Retinol			-	5	5	-
Myristoyl Tetrapeptide-34			-	-	10	10

(ppm)

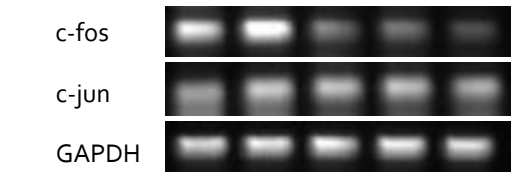
DermaPep™ A440
In vitro efficacy

Signaling of MMP-1 expression

Hs68 cells were seeded into 6-well plates and pretreated with the retinoic acid or Myristoyl Tetrapeptide-34 for 24 and then stimulated by UV irradiation. After 30 minutes, total cellular RNA was extracted and reverse-transcription PCR was performed to determine c-jun and c-fos mRNA level. β-actin was used for sample standardization.

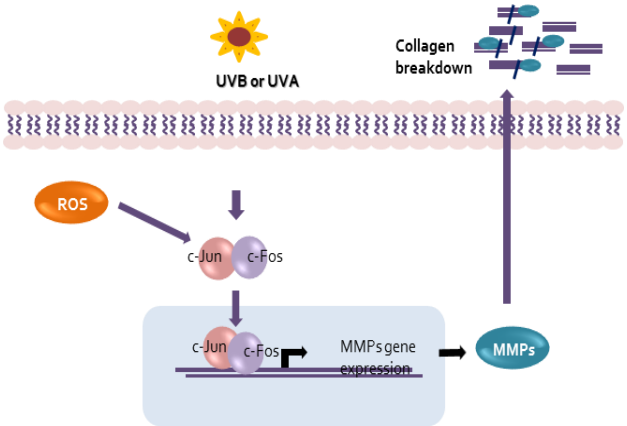
Myristoyl Tetrapeptide-34 significantly decreased UVB induced c-fos mRNA expression

AP-1 component gene expression(PCR)

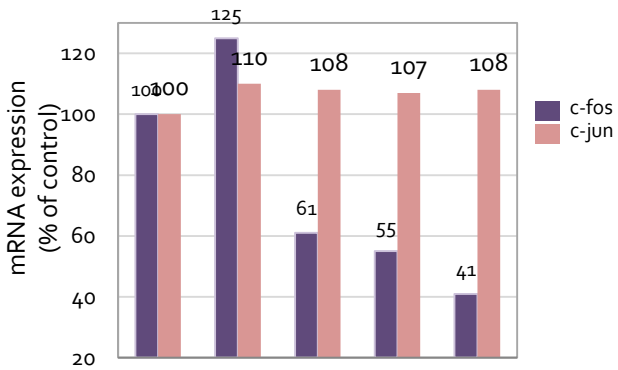


UV	-	+	+	+	+
Myristoyl Tetrapeptide-34			5	10	20 (ppm)

Mechanism of MMP-1 expression



c-fos/c-Jun gene expression



UV	-	+	+	+	+
Myristoyl Tetrapeptide-34			5	10	20 (ppm)

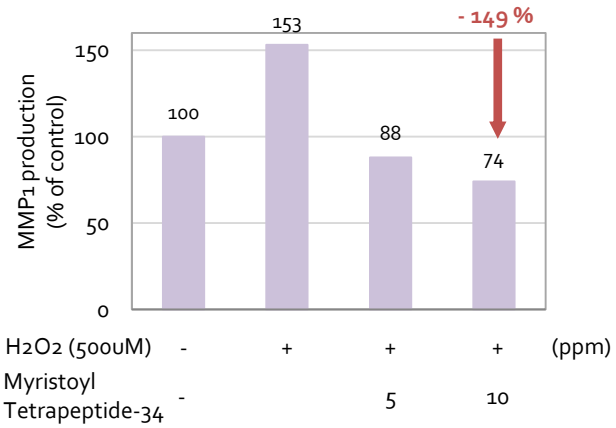
DermaPep™ A440
In vitro efficacy

Inhibition of ROS Induced MMP-1 Production and AP-1 Signaling

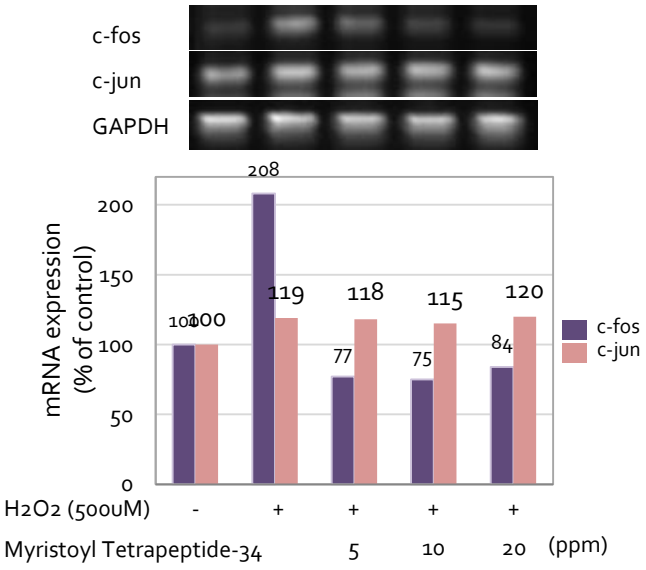
Hs68 cells were seeded into 6-well plates and treated with Myristoyl Tetrapeptide-34 followed by or H_2O_2. Equal amounts of media were analyzed by ELISA assay for MMP-I. Cell viability was used for sample standardization. For AP-1 signaling, total cellular RNA was extracted and reverse-transcription PCR was performed to determine c-jun and c-fos mRNA level. GAPDH was used for sample standardization.

Myristoyl Tetrapeptide-34 significantly decreased ROS radical stimulated MMP-1 production and c-fos mRNA expression.

Inhibition of MMP-1 production against H_2O_2



AP-1 component gene expression(PCR)



DermaPep™ A440

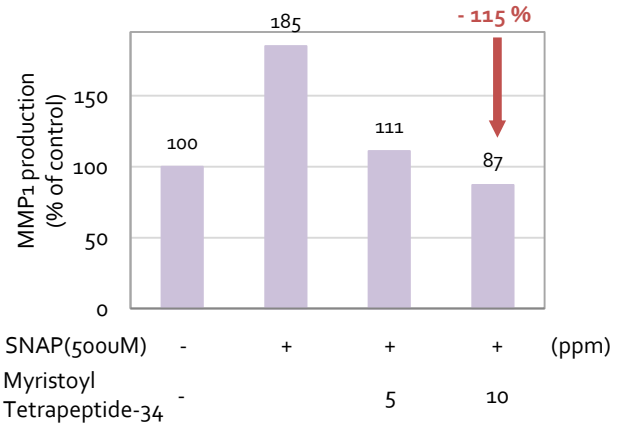
In vitro efficacy

Inhibition of RNS Induced MMP-1 Production and AP-1 Signaling

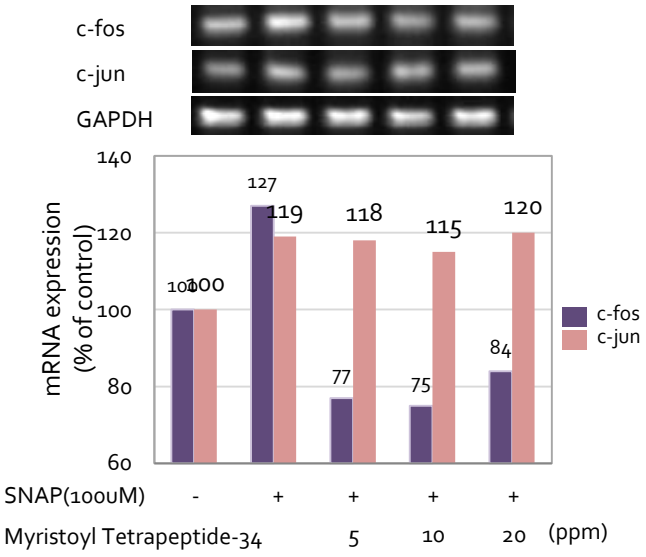
Hs68 cells were seeded into 6-well plates and treated with the Myristoyl Tetrapeptide-34 followed by SNAP(S-nitroso-N-acetylpenicillamine. Equal amounts of media were analyzed by ELISA assay for MMP-I. Cell viability was used for sample standardization. For AP-1 signaling, total cellular RNA was extracted and reverse-transcription PCR was performed to determine c-jun and c-fos mRNA level. GAPDH was used for sample standardization.

Myristoyl Tetrapeptide-34 significantly decreased RNS radical stimulated MMP-1 production and c-fos mRNA expression.

Inhibition of MMP-1 production against SNAP



AP-1 component gene expression(PCR)



DermaPep™ A440
In vitro efficacy

Anti-inflammatory Effects of Myristoyl Tetrapeptide-34 on Human Fibroblasts

Hs68 cells were seeded into 6-well plates and pretreated with TGF-β, retinoic acid or Myristoyl Tetrapeptide-34 followed by 30J/cm² UVA . After 24 hours, total cellular RNA was extracted and reverse-transcription PCR was performed to determine IL-1β, IL-6, IL-8 . GAPDH mRNA level was used for sample standardization.

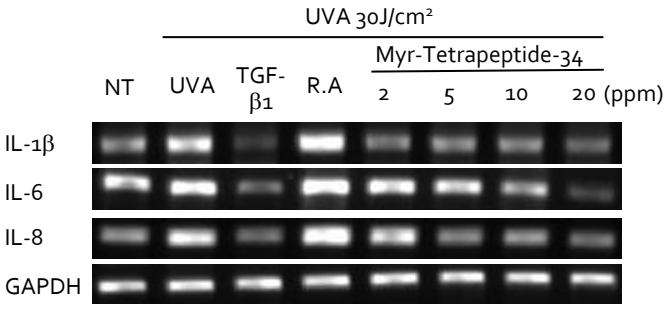
Myristoyl Tetrapeptide-34 greatly inhibited UVA-induced expression of pro-inflammatory cytokines in human skin fibroblasts.

Anti-inflammatory Effects of Myristoyl Tetrapeptide-34 on Keratinocytes

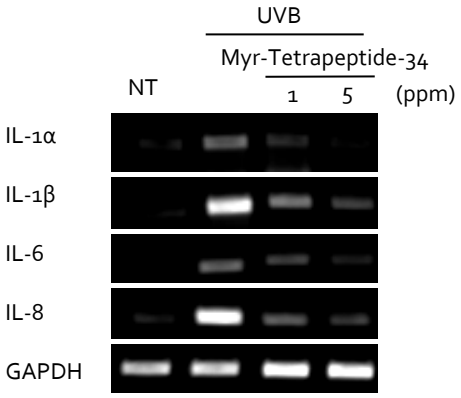
Human keratinocyte HaCaT cells were treated with Myristoyl Tetrapeptide-34 and then stimulated with UVB (100 mJ/cm²). After 24 hours, total cellular RNA was extracted and reverse-transcription PCR was performed to determine IL-1α, IL-1β, IL-6. GAPDH mRNA level was used for sample standardization.

Myristoyl Tetrapeptide-34 significantly inhibited UVB-induced expression of pro-inflammatory cytokines in human keratinocytes.

Pro-inflammatory cytokines Level (Hs68)



Pro-inflammatory cytokines Level (HaCaT)



DermaPep™ A440
In vitro efficacy

DNA Chip Assay

CHIP data of pro-inflammatory related gene

Index	ProbeName	Description	Gene Symbol	Genbank Accession	UniGeneID	A440/UV intensity
1078	A_23_P121253	tumor necrosis factor (ligand) superfamily, member 10 (TNFSF10), transcript variant 1, mRNA	TNFSF10	NM_003810	Hs.478275	0.712051591
2139	A_23_P14174	tumor necrosis factor (ligand) superfamily, member 13b (TNFSF13B), transcript variant 1, mRNA	TNFSF13B	NM_006573	Hs.525157	0.444733865
7521	A_23_P376488	tumor necrosis factor (TNF), mRNA	TNF	NM_000594	Hs.241570	0.679159864
3365	A_23_P165624	tumor necrosis factor, alpha-induced protein 6 (TNFAIP6), mRNA	TNFAIP6	NM_007115	Hs.437322	0.542426693
10479	A_23_P71530	tumor necrosis factor receptor superfamily, member 11b (TNFRSF11B), mRNA	TNFRSF11B	NM_002546	Hs.81791	0.50666134
4776	A_23_P218646	tumor necrosis factor receptor superfamily, member 6b, decoy (TNFRSF6B), mRNA	TNFRSF6B	NM_003823	Hs.434878	0.587013243
25168	A_33_P3305571	tumor necrosis factor receptor superfamily, member 6b, decoy (TNFRSF6B), mRNA	TNFRSF6B	NM_003823	Hs.434878	0.589897775
1337	A_23_P126836	tumor necrosis factor (ligand) superfamily, member 4 (TNFSF4), mRNA	TNFSF4	NM_003326	Hs.181097	0.858187786
21238	A_33_P3246833	interleukin 1 receptor antagonist (IL1RN), transcript variant 4, mRNA	IL1RN	NM_173843	Hs.81134	0.398674612
12985	A_24_P200023	interleukin 1 receptor, type I (IL1R1), mRNA	IL1R1	NM_000877	Hs.701982	0.547208757
31154	A_33_P3396389	interleukin 1 receptor, type I (IL1R1), mRNA	IL1R1	NM_000877	Hs.701982	0.654729398
10576	A_23_P73780	interleukin 1 receptor-associated kinase 1 (IRAK1), transcript variant 1, mRNA	IRAK1	NM_001569	Hs.522819	0.826588326
11156	A_23_P85209	interleukin 13 receptor, alpha 2 (IL13RA2), mRNA	IL13RA2	NM_000640	Hs.336046	0.454015552
1964	A_23_P138680	interleukin 15 receptor, alpha (IL15RA), transcript variant 2, mRNA	IL15RA	NM_172200	Hs.524117	0.696403722
5072	A_23_P252062	peroxisome proliferator-activated receptor gamma (PPARG), transcript variant 3, mRNA	PPARG	NM_138711	Hs.162646	0.620602594
28110	A_33_P3350726	peroxisome proliferator-activated receptor gamma (PPARG), transcript variant 3, mRNA	PPARG	NM_138711	Hs.162646	0.587618207

CHIP data of ECM protein related gene

Index	ProbeName	Description	Gene Symbol	Genbank Accession	UniGeneID	UV/NT intensity	A440/UV intensity
25105	A_33_P3304668	collagen, type I, alpha 1 (COL1A1), mRNA	COL1A1	NM_000088	Hs.172928	0.445770446	1.932809889
11097	A_23_P83818	collagen, type V, alpha 1 (COL5A1), mRNA	COL5A1	NM_000093	Hs.210283	0.749845964	1.621188441
33475	A_33_P3629678	collagen, type V, alpha 1 (COL5A1), mRNA	COL5A1	NM_000093	Hs.210283	0.559161349	1.618872875
31278	A_33_P3398236	collagen, type XXVIII, alpha 1	COL28A1	AJ890452	Hs.491104	0.934087883	3.457301057
14419	A_24_P334130	fibronectin 1 (FN1), transcript variant 7, mRNA	FN1	NM_054034	Hs.203717	0.850157729	2.353647741
2910	A_23_P156327	transforming growth factor, beta-induced, 68kDa (TGFBI), mRNA	TGFBI	NM_000358	Hs.369397	0.682829566	2.148446928
25707	A_33_P3313825	transforming growth factor, beta receptor II (70/80kDa)	TGFBR2	AJ786388	Hs.82028		1.62420191
15179	A_24_P402438	transforming growth factor, beta 2 (TGFB2), transcript variant 2, mRNA	TGFB2	NM_003238	Hs.133379	0.971181556	1.69905287
8176	A_23_P405129	latent transforming growth factor beta binding protein 2 (LTBP2), mRNA	LTBP2	NM_000428	Hs.512776	1.024822695	2.42692819
3781	A_23_P19663	connective tissue growth factor (CTGF), mRNA	CTGF	NM_001901	Hs.410037	0.332717584	3.151554741
30141	A_33_P3380797	fibroblast growth factor 3 (FGF3), mRNA	FGF3	NM_005247	Hs.37092	0.965838192	2.016988657
3838	A_23_P200741	dermatopontin (DPT), mRNA [NM_001937]	DPT	NM_001937	Hs.80552	0.762254902	1.327178047
13766	A_24_P274270	signal transducer and activator of transcription 1, 91kDa (STAT1), transcript variant beta, mRNA	STAT1	NM_139266	Hs.642990	2.028586279	0.346110016
9975	A_23_P62115	TIMP metallopeptidase inhibitor 1 (TIMP1), mRNA	TIMP1	NM_003254	Hs.522632	0.850732951	1.517430948
3547	A_23_P1691	matrix metallopeptidase 1 (interstitial collagenase) (MMP1), transcript variant 1, mRNA	MMP1	NM_002421	Hs.83169	1.564405238	0.568818868
3181	A_23_P161698	matrix metallopeptidase 3 (stromelysin 1, progelatinase) (MMP3), mRNA	MMP3	NM_002422	Hs.375129	2.803817166	0.82164818
16187	A_24_P82106	matrix metallopeptidase 14 (membrane-inserted) (MMP14), mRNA	MMP14	NM_004995	Hs.2399	1.135234216	0.695241922

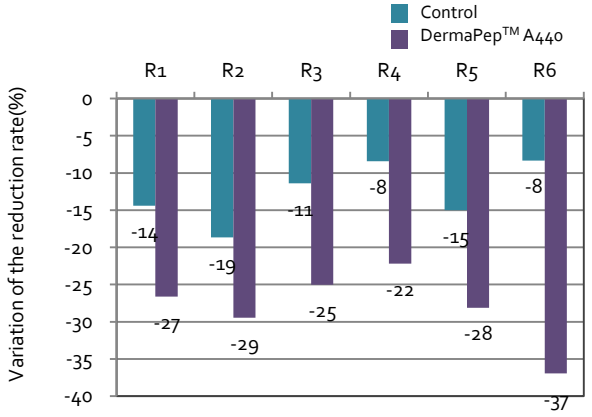
DermaPep™ A440
In vivo efficacy

Anti-Aging Efficacy on Asian Skin Type

In a clinical study with 21 Asian healthy female volunteers (Korea), aged 30 to 65, 2 % formula containing DermaPep™A440 had been applied twice daily during 12 weeks on the face area. Evaluation was performed by Transparancy profilometry analysis using visiometer SV600(Courage-Khazaka electronic GmbH, Germany) and digital photography.

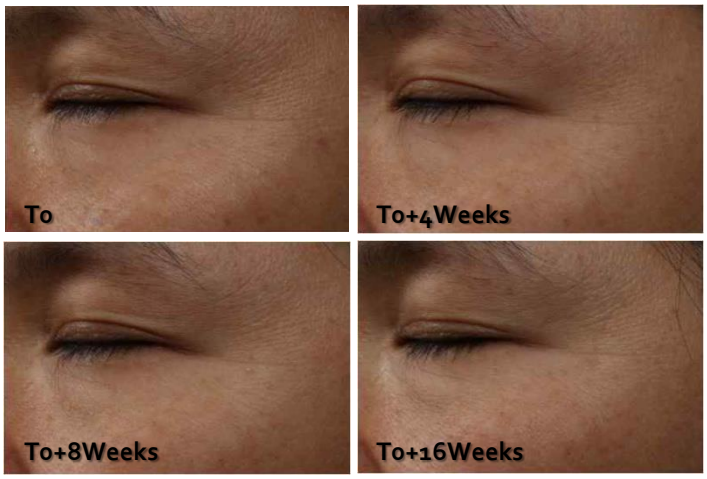
DermaPep™A440 showed significant improvement in all the parameters measured and proved to be the powerful anti-wrinkle agent.

Anti aging effect



R1: Skin roughness
R2: Maximum roughness
R3: Average roughness
R4: Smoothness depth
R5: Arithmetic average roughness
R6: Expert's assessment

Illustrative Picture_Code No.11915-K1-12



2% DERMAPEP™ A440				
Code 11915-K1-12	T0	T+4 weeks	T+8 weeks	T+12 weeks
Skin Roughness	134.2	93.08	99.35	63.18
Maxium Roughness	90.13	66.23	68.86	49.11
Average Roughness	53	48.03	45.6	35.5
Smoothness Depth	62.31	36.36	38.47	26.45
Arithmetic average Roughness	31.24	13.62	17.04	9.74
Experts assessment	3	3	2	1

Miwon Commercial Co., Ltd.
Personal Care Ingredients Business Unit
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Last revised on Apr 17^{h}, 2013