In Vitro Skin Anti Wrinkle Effect Test Report	
Record/Report No	2025.05.15E
Report Date	15.05.2025
Test Start-End Date	08.05.2025 – 14.05.2025
Requesting Company/Person	Akra Group Kozmetik ve İlaç San. Tic. A.Ş.
Requesting Company Address	Yakuplu Mh. 228. Sk. No:18 İç Kapı: 3 Beylikdüzü/İstanbul
Product Name	**Duaderm Barrier Cream**
Requested Tests	Anti Wrinkle
Sonuç Yorum	The test material has anti- wrinkle effect.
Additional	Test Report

Approved by the Report
Dr. OĞUZ ÖZTÜRK

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In Vitro Skin Anti-Wrinkle Effect Test Report	
1.	Test Material Information
2.	Test Information
3.	Test Result

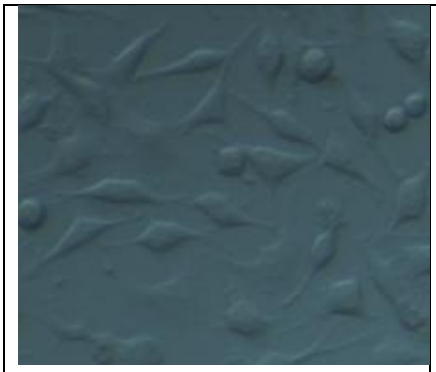
1	**Test Material Information (TM):** Duaderm Barrier Cream **Lot Number:** 1235-01
2	**Test information** Type I collagen is the most abundant protein found in all vertebrates. It is a simple and fibrillar scleroprotein found in significant amounts in tendons, cartilage, the organic matrix of bones, and the cornea of the eye. Collagen is mainly synthesized by fibroblasts, myofibroblasts, osteoblasts, and chondrocytes (Ganceviciene et al., 2012). Anti-aging creams used against the decrease in collagen I with age can increase the amount of collagen I. For this purpose, the amounts of collagen I were measured in the test (Kramer et al., 2001). **Cell Culture Conditions** In all experiments within the scope of the study, human skin fibroblast cell line HS68 (ATCC CRL-1635) obtained from ATCC was used. Cells were propagated in DMEM [Dulbecco's Modified Eagle's Medium (ATCC Cat No: 30-2006)] supplemented with 10% FBS and 2% glutamine and incubated at 37°C in a 5% CO_2 incubator. A mixture of 0.25% trypsin and 0.03% EDTA was used for trypsinization of cells as recommended by ATCC. Cells were divided into 6-well plates as $2x10^5$ cells per well. The amounts of Collagen αI (Col I) released from HS68 cells in the medium were determined using the Human Collagen αI ELISA Kit at the end of 48 hours of incubation. Before starting the experiment, each well was washed four times with 300 µL of 1x wash buffer. 50 µL from each experimental group and control group, which were applied with the determined doses of test material, were added to these wells. They were incubated for two hours at room temperature on a shaker at 200 rpm. Each well was washed four times with 300 µL of 1x wash buffer. Then, 100 µL of Human Col I antibody was added to each well. It was incubated for one hour at room temperature on a shaker. Each well was washed four times with 300 µL of 1x wash buffer. 100 µL of Avidin-HRP A (Avidin Peroxidase A) solution was added to each well and incubated for 30 minutes at room temperature on a shaker.. Each well was washed five times with 300 µL of 1x wash buffer. 100 µL of Substrate F (high sensitivity TMB) solution was added to each well and incubated for 10 minutes at room temperature and in the dark. Afterwards, blue color formation was observed depending on the amount of Col I bound to the wells. The reaction was stopped by adding 100 µL of stop solution to each well and the color changed from blue to yellow. Absorbance values of the samples were read at 450 nm in the Eliza kit reader (Thermo Fisher, Multi Scan FC Microplate Reader). **Test Product Extract Preparation Protocol**

Negative Control (NC): DMEM	As a negative control, medium containing only DMEM was used in the cell wells.
Positive Control (PC): Ellagic Acid	2mg of ellagic acid has a molecular weight of 302.192 g/mol and was dissolved in 1324 µl DMSO (Dimethyl sulfoxide). The stock solution was diluted with DMEM to be 100µM. It was applied as a positive control group.
Test Material Extract Preparation	The test material was dissolved in DMEM medium as 0.1 gr/ml. It was incubated for 24 hours at 37 °C in a shaking incubator at 120 rpm. At the end of the period, it was passed through a 0.22 µm membrane filter and

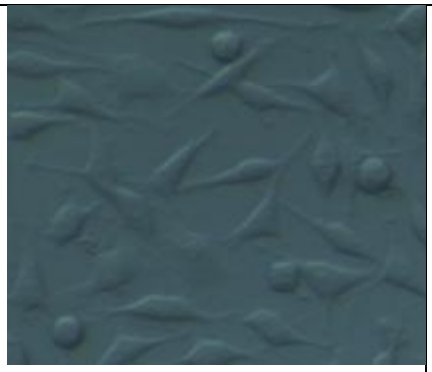
	used in test concentrations. Extraction was performed according to the ISO 10993-12 test standard.. **Test Material Application doses (w/v):** 100 and 200 µg/ml

Microscopic Imaging

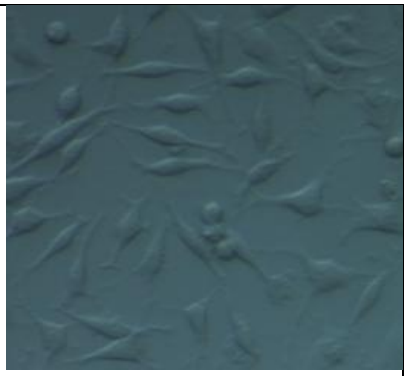
Figure 1. A. Negative Control **B**. Positive Control **C.** Microscopic images of 200 µg/ml concentration of test material



A



B



C

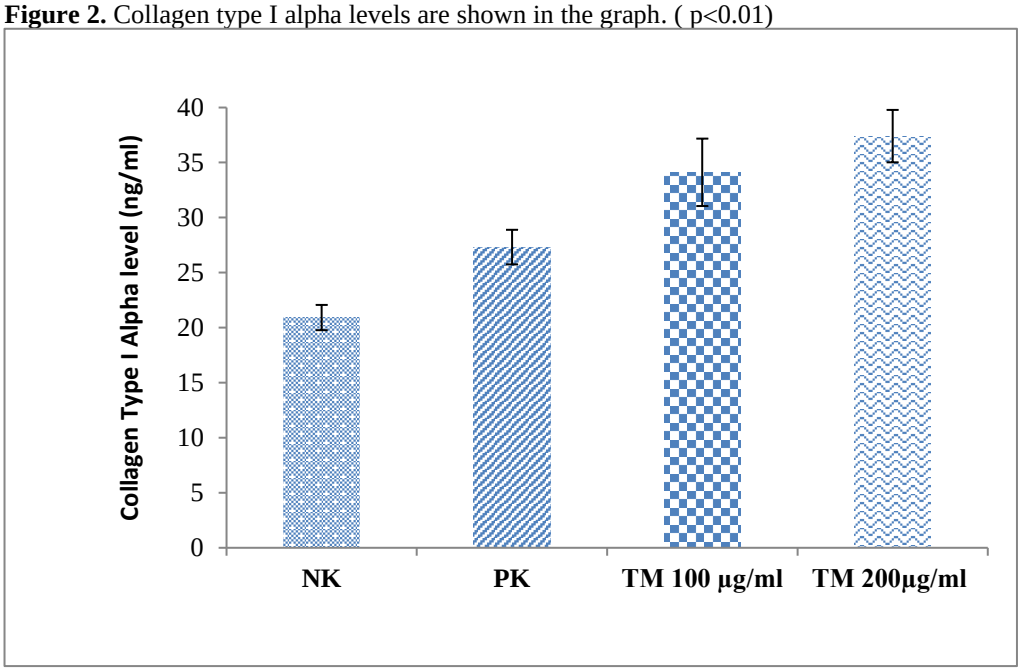
3 Anti Wrinkle Test Result

Experiments were performed in 5 repetitions and the results were given as Mean ± Standard Deviation (SD). Statistical analysis was performed with SPSS Version 23 computer program. Differences between negative control and other groups were measured with one-way analysis of variance (ANOVA) and differences between groups were measured with POST HOC analysis. P values less than 0.01 were considered statistically significant..

Table 1. Showing the effect of test material on Collagen type I alpha levels [n.s (non-significant) *p<0.01].

Collagen 1A level (ng/ml) ± SD (Standard Deviation)				
N=5	***NC***	***PC***	***TM 100 µg/ml***	***TM 200 µg/ml***
1	22,16	27,16	34,12	36,25
2	21,45	26,45	38,36	34,16
3	20,29	25,29	30,25	37,6
4	19,25	29,25	32,42	40,5
5	21,44	28,44	35,41	38,5
Average ±SD	*20,91±1,14*	*27,32±1,57**	*34,11±3,06*$^{n.s}$	*37,40±2,38**

Figure 2. Collagen type I alpha levels are shown in the graph. (p<0.01)



As a result of the test, it was determined that there was a statistical increase in collagen type I alpha levels in100 and 200 µg/ml concentrations of **DUADERM BARRIER CREAM** product compared to the control group. There is a statistical difference in the 100 and 200 µg/ml concentration compared to the positive control. According to these results, the Test Material is an effective anti -wrinkle product..

APPROVAL . 15.05.2025

DR. OĞUZ ÖZTÜRK

Resources

Dorjsembe B, Lee HJ, Kim M, Dulamjav B, Jigjid T, Nho CW., 2017. Achillea asiatica extract and its active compounds induce cutaneous wound healing. J Ethnopharmacol. 12;206:306-314.

Ganceviciene, R., Liakou, A.I., Theodoridis,A., Makrantonaki, E. and Zouboulis C.C., 2012, Skin anti-aging strategies, Dermatoendocrinol.4(3),308-19p.

Hseu YC, Korivi M, Lin FY, Li ML, Lin RW, Wu JJ, Yang HL.,2018. Trans-cinnamic acid attenuates UVA-induced photoaging through inhibition of AP-1 activation and induction of Nrf2-mediated antioxidant genes in human skin fibroblasts. J Dermatol Sci. 90(2):123-134.

Kramer, R. Z., Bella, J., Brodsky, B. and Berman, H. M., 2001, The crystal and molecular structure of a collagen-like peptide with a biologically relevant sequence, J Mol Biol, 311 (1), 131-147p.

Lee K, Kim HJ, Kim SA, Park SD, Shim JJ, Lee JL.,2021. Exopolysaccharide from *Lactobacillus plantarum* HY7714 Protects against Skin Aging through Skin-Gut Axis Communication. Molecules.16;26(6):1651.

Vafa BR , Vahid RA , Seyed HR., 2018.Ellagic acid reveals promising anti-aging effects against D-galactose-induced aging on human neuroblastoma cell line, SH-SY5Y: A mechanistic study. *Biomedicine & Pharmacotherapy.* 108(5):1712-1724.

Rebeca R, Manual RF., et all., 2023. Effect of Punicalagin and Ellagic Acid on Human Fibroblasts In Vitro: A Preliminary Evaluation of Their Therapeutic Potential**.** Nutrients. 20 (16).1