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In Vitro Skin Anti Aging Effect Test Report

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Requesting Company	Teksel Tekstil Ürünleri Pazarlama San. Ve Tic A.Ş
Requesting Company Adress	15 Temmuz Mah. Bahar. Cad. No:6 Polat İs Mrkz. C Blok D41 K4 Bağcılar-İSTANBUL
Product Name	Umorphyl & Silver Blended Yarn
Requested Test	Anti Aging Test
Result and Comment	The test material has a anti aging effect
Additional	Test report

Approved by:

Dr. OĞUZ ÖZTÜRK

Biochemistry Specialist

R & D

The findings and conclusions of the report are from the material tested. This report is 3 pages and has been prepared as 2 originals (1 main customer, 1 main corporate archive).

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1.	Test Material Information
2.	Test Information
3.	Test Result

1: **Test Material Information (TM)** : Umorphyl & Silver Blended Yarn 60% Umorphyl, 35% Cotton, 5% Silver

Product Date: Unspecified. **Sample Qantity:** 20 g / 2 pcs-piece

Test Material Picture

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, organic matrix of bones, and 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 the amount of collagen I with age can increase the amount of collagen I. For this purpose, the amount of collagen I was measured in the test (Kramer et al., 2001).

Cell Culture Conditions

Human skin fibroblast cell line HS68 (ATCC CRL-1635) from ATCC was used in all experiments in the study. Cells were grown in DMEM (ATCC Cat No: 30-2006) supplemented with 10% FBS and 2% glutamine and incubated at 37 ° C in a 5% CO 2 oven. A mixture of 0.25% trypsin and 0.03% EDTA was used for trypsinasis of cells as suggested by ATCC.

After centrifugation by trypsination, 10 mL of DMEM medium containing 10% FBS was added to the cells obtained in the falkon tube and mixed well. Then, 900 ml of 10% DMEM medium was added to the ependorf tube and 100 indenL of the cell containing medium was diluted 10 times. This cell mixture was counted on the Thoma slide. The number of cells in these areas was averaged in the Thoma slide with four 16 squares. This average value was multiplied by the dilution coefficient and 10^4 to obtain the number of cells per mL. Cells were divided into 6-well plates at $2x10^5$ cells per well. The amounts of Collagen αI (Col I) released from the HS68 cells were determined using the Human Collagen αI ELISA Kit after 48 hours of incubation.

The kit contains a 96-well plate coated with a Col I-specific monoclonal antibody. Before starting the experiment, each well was washed four times with 300 ml of 1x wash buffer (Tween 20 and PBS mixture). 50 ml of each test group and control group were applied to the wells. Incubate for two hours at 200 rpm on

a shaker at room temperature. Each well was washed four times with 300 LL of 1x wash buffer. 100 ml of Human Col I antibody was then added to each well. It was incubated for one hour at room temperature with shaker. Each well was washed four times with 300 ml 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 with shaker.Each well was washed five times with 300 ml of 1x wash buffer. 100 ml of Substrate F (high precision TMB) solution was added to each well and incubated for 10 minutes at room temperature and in the dark. Subsequently, blue color formation was observed depending on the amount of Col I bound to the wells. The reaction was stopped by adding 100 ml of stop solution to each well and the color changed from blue to yellow. Absorbance values of the samples were read in the Eliza kit reader (Thermo Fisher, Multi Scan FC Microplate Reader) at 450 nm.

Negative Control (NC): Ultra Pure Water	**Test Material Application Doses (w/v):** 25 µg/ml, 50 µg/ml,100 and 200 µg/ml
Test Material Extraction Conditions	The test material was dissolved in DMEM medium containing 0.05% DMSO as 0.1gr/ml. It was incubated for 24 hours at 37 °C in a shaking incubator with 120 rpm. At the end of the period, it was passed through a 0.22 µm membrane filter and used at test concentrations. Extraction was carried out according to the ISO 10993-12 test standard.

3 **Test Results**

Experiments were repeated as 5 times and the results were given as mean ± standard deviation. Comparisons between the control group were made with One-way Analysis of Variance (ANOVA).

Table 1. Demonstration of the effect of test material on Collagen Type I Alpha levels [n.s (non-significant) *p<0.05, **p<0.01].

Collagen Type 1A Levels (ng/ml) ± SD (Standard Deviation)				
NC	***TM 25 µg/ml***	***TM 50 µg/ml***	***TM 100 µg/ml***	***TM 200 µg/ml***
26,95±2,21	**27,48 ±4,46n.s**	**28,01±2,79n.s**	**30,54 ±5,42ns**	**34,46± 6,26***

As a result of the test, it was determined that there was a statistical increase in collagen type I alpha levels compared to the control group at 200µg/ml concentrations of 60 Umorphil, 35% Cotton, 5% Silver blended Yarn products. **According to these results, the Test Material is an effective anti-aging product.**

CERTIFIED. 29.11.2022

Dr. OĞUZ ÖZTÜRK

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