Cell Proliferation Test Report

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Requesting Company	Akra Group Kozmetik ve İlaç San. Tic. A.Ş.
Requesting Company Adress	Yakuplu Mh. 228. Sk. No:18 İç Kapı: 3 Beylikdüzü/İstanbul
Product Name	Duaderm Barrier Cream
Requested Test	Cell Proliferation Test Report
Result and Comment	The test material has a cell proliferation effect
Additional	Test report

Approved by the Report
Dr. OĞUZ ÖZTÜRK

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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Cell Proliferation Test Report

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

1 **Test Material Information (TM)** : Duaderm Barier Cream

Lot Number: 1235-01

2 **Test Information**

Proliferation test is a test method that is measured by detecting the binding of BrdU (5-bromodeoxyuridine) to DNA in cells (ROCHE, 2012). BrdU is replaced by thymidine in the DNA of proliferating cells. It is based on BrdU labeling of the S phase cells and monitoring the progression of this labeled cell group in the cell cycle.

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.

Dose screening was performed to examine the effects of test material on cell proliferation. HS68 cells were divided into 96-well sterile plates at 5x10 ^{3} cells / well and incubated for 24 hours to adhere. At the end of the incubation period, the media of the cells were removed and different concentrations of test material were applied to each well to 100 konsantrasyon / well. It was incubated for 72 hours. After the addition of anti-BrdU-peroxidase antibody, immune complexes were measured by spectrophotometer at 370 nm.

Negative Control (NC): Ultra pure water
Doses (v / v) 50, 100,200 µg / ml

Test material dissolved in 0.05% DMSO compound

3 Test Result

With the Roche brand Cell Proliferation Elisa BrdU (colorimetric) Version 16 kit, measurements were made by repeating each concentration 5 times. Instad 10.0 statistical program was evaluated with Student-Newman-Keuls Multiple Comparisons Test. Results are given as mean ± standard error (SEM).

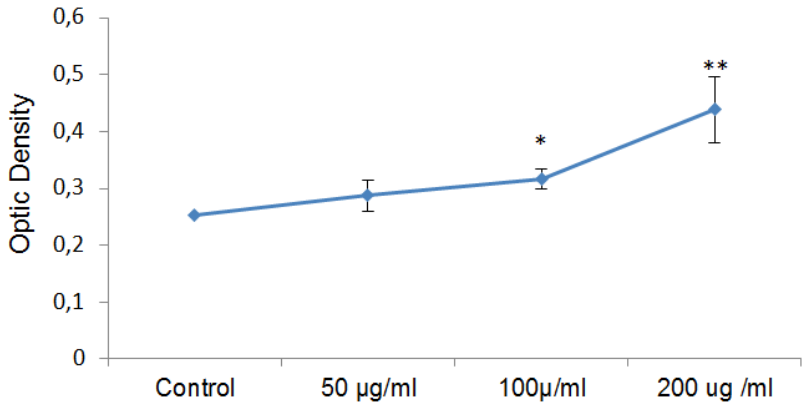


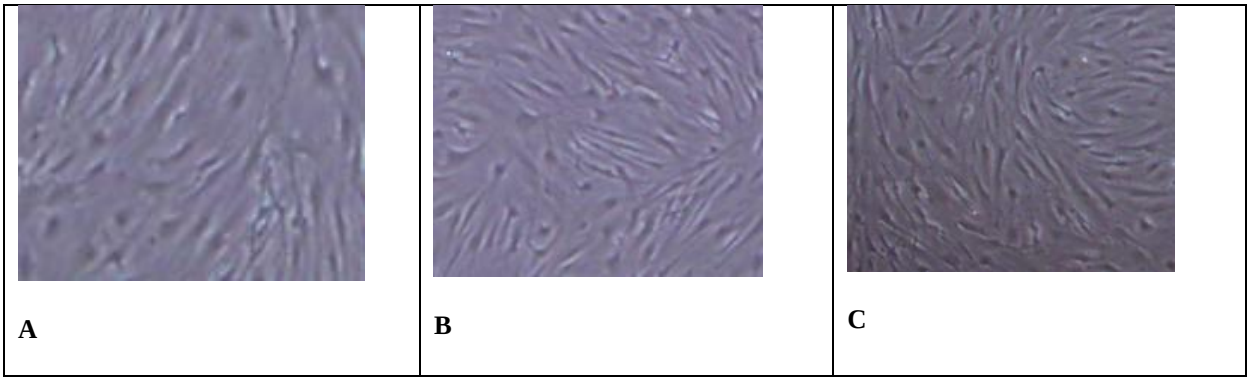
Figure 1. Optical density measurements plot of proliferative property of test material.

	Optic Density (OD)		
Kontrol	**50µg/ml**	**100 µg/ml**	**200 µg/ml**
0,25±0,009	0,28±0,027	0,317±0,017*	0,43±0,058**

Table 1. Demonstration of the effect of test material on cell proliferation.

Microscopic Imaging

Figure 2 1. A. Negative Control B. Positive Control (Ellagic Acid) C. Microscopic images of 200 µg/ml concentration of test material



A **B** **C**

As a result of the test, it was found that proliferative effect (cell renewal) increased when compared with the control group with the application of **Duaderm Barrier Cream** 100 and 200 µg / ml. According to these results, **Duaderm Barrier Cream** is a proliferative effective product.

APPROVAL 15.05.2025

DR. OĞUZ ÖZTÜRK

References:
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