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FINAL REPORT

MEM ELUTION

PROCEDURE NO. STP0032 REV 03

LABORATORY NO. 452240

ULTIMATE SKIN & BODY CARE

CYTOTOXICITY TEST

PREPARED FOR:

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LABORATORY NUMBER:	452240
PROCEDURE NUMBER:	STP0032 REV 03
SAMPLE SOURCE:	American Biotech Labs
TYPE OF TEST:	Solid
SAMPLE IDENTIFICATION:	Refer to Table 1
DEVIATIONS:	None
CELL LINE:	Mouse Heteroploid Connective Tissue (L-929)
INCUBATION PERIOD:	72 ± 3 hours at 37 ± 1°C
METHOD OF SCORING:	Cytopathic Effect (0-4)
AMOUNT TESTED/SAMPLE EXTRACT:	3 g / 15.0 mL
SAMPLE RECEIVED DATE:	21 Nov 2008
LAB PHASE START DATE:	24 Nov 2008
LAB PHASE COMPLETION DATE:	29 Nov 2008
REPORT ISSUE DATE:	01 Dec 2008

INTRODUCTION:

The MEM Elution test was designed to determine the cytotoxicity of extractable substances. An extract of the sample was added to cell monolayers and incubated. The cell monolayers were examined and scored based on the degree of cellular destruction.

ACCEPTANCE CRITERIA:

The United States Pharmacopeia & National Formulary states that the sample meets the requirements if the reactivity grade is not greater than grade 2 or a mild reactivity. The AAMI/ISO 10993-5 standard states that the overall assessment of the results shall be made by capable persons based upon the data and results. Nelson Laboratories acceptance criteria was based upon the negative and media controls receiving "0" reactivity grades and positive results receiving a 3-4 reactivity grades (moderate to severe).

PROCEDURE:

The amount of test material extracted was based on ANSI/AAMI/ISO and USP surface area recommendations or weight (0.20 g/mL extract fluid for polymers and plastic). The sample was extracted for 24-25 hours at 37 ± 1°C in 1X Minimal Essential Media with 5% calf serum. Positive (Latex Natural Rubber) and negative (Polypropylene Pellets) controls were extracted and included in the assay. A blank of extraction media (media control) was also included in the assay.

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Multiple well cell culture plates were seeded with a verified quantity of L-929 cells and incubated until 80-90% confluent. The cell culture media was removed from the plates. The test extracts were filtered and the appropriate amount of extract was added to each well on the cell culture plates. Each extract was tested on three wells of cells. The cells were incubated at 37 ± 1°C with 5 ± 1% CO_2 for 72 ± 3 hours.

The cell monolayers were examined microscopically. The wells were scored as to the degree of discernable morphological cytotoxicity on a relative scale of 0 to 4:

CONDITIONS OF ALL CULTURES	REACTIVITY	GRADE
No cell lysis, intracytoplasmic granules.	NONE	0
Not more than 20% rounding, occasional lysed cells.	SLIGHT	1
Not more than 50% rounding, no extensive cell lysis.	MILD	2
Not more than 70% rounding and lysed cells.	MODERATE	3
Nearly complete cell destruction.	SEVERE	4

The results from the three wells were averaged to give a final cytotoxicity score.

RESULTS:

The results are summarized in Table 1. The test is acceptable if all three of the negative control and medium control test wells have a score of 0 and all three of the positive control test wells have a score of 3 or higher.

The sample meets USP requirements if none of the cell culture exposed to the sample shows greater than a mild reactivity (grade 2).

STATEMENT OF UNCERTAINTY:

If applicable, the statement of uncertainty is available to sponsors upon request.

Technical Reviewer

Bobbi Rushton-Castro
Study Director

01 Dec 2008
Study Completion Date

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TABLE 1. Results

IDENTIFICATION	SCORE #1	SCORE #2	SCORE #3	AVERAGE
Negative Control	0	0	0	0
Media Control	0	0	0	0
Positive Control	4	4	4	4
ASAP Ultimate Skin and Body Care Lot #08295	1	1	1	1