

Mr. Patrick Robb
Stratasys, Inc.
7665 Commerce Way
Eden Prairie, MN 55344

Fax #:
Phone #: 952-906-2767

140320 : MEM Elution Using L-929 Mouse Fibroblast Cells (ISO) (Cytotoxicity)

Test Article: PC-ISO (White)

Lot #: NA

Exp. Date: NA

Sterilization Method: Non-Sterile

Project Number 145176

Test Start Date 10/18/10

Date Received 10/14/10

Completion Date 10/22/10

Purchase Order 147357

Date Reported 10/22/10

Purpose

The purpose of this study was to evaluate the ability of a test article extract to elicit a cytotoxic response in cultured cells. L-929 Mouse Fibroblast Cells were employed for this assay by a method compliant with the requirements specified in ISO 10993-5; 2009.

Test Article Preparation

The test article was prepared using an extraction ratio of 60 cm² / 20 mL. 15.0 cm² of test article was extracted in 5.0 mL of Eagle's Minimum Essential Medium (E-MEM) + 5% FBS.

Experimental Design

Samples were extracted at 37 ± 1 °C for 24-25 hours. The extract was inoculated onto the cell line and incubated at 37 ± 1 °C in a humidified atmosphere with 5 ± 1% CO₂ in the air. Positive, intermediate and negative controls were run in parallel with the test article. Cultures were evaluated for cytotoxic effects by microscopic observation after 24, 48 and 72 hour incubation periods.

Validity Criteria

The positive control should display a moderate to strong cytotoxic reaction, displaying a score of "3" or "4" and the negative control should maintain a healthy normal appearance throughout the duration of the test for a valid assay. If the suitability of the test cannot be confirmed then the test may be repeated.

Results

Criteria for evaluating cytotoxicity included morphologic changes in cells, such as granulation, crenation, or rounding, and loss of viable cells from the monolayer by lysis or detachment. The cytotoxicity scores for the test article and controls are displayed in Table 1. The reactivity grades for the MEM Elution Test are given in Table 2. According to USP, test articles scoring "0", "1" or "2" are considered NON-TOXIC. Test articles scoring "3" or "4" are considered TOXIC.

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Table 1. Test Article and Control Results

Test Article and Controls	Cytotoxicity Scores		
	24 Hours	48 Hours	72 Hours
Test Article	0/0/0	0/0/0	0/0/0
Positive Control - 100 uM CdCl ₂	4/4/4	4/4/4	4/4/4
Intermediate Control - Black Rubber Stopper	2/2/2	2/2/2	2/2/2
Negative Control - HDPE	0/0/0	0/0/0	0/0/0
Cell Control - L-929 Mouse Fibroblast Cells	0/0/0	0/0/0	0/0/0

Table 2. Reactivity Grades for MEM Elution Test


Score	Reactivity	Conditions of All Cultures
0	None	Discrete intracytoplasmic granules; no cell lysis
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules; no extensive cell lysis and empty areas between cells
3	Moderate	Not more than 70% of the cells are rounded or lysed
4	Severe	Nearly complete destruction of the cell monolayer

Analysis and Conclusion

The test was considered valid as the control results were within acceptable parameters. The test article PASSES and is considered NON-TOXIC under the test conditions employed.

Reviewed and Released by: amanda.lien

Date: 10/22/2010

 10/22/10



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