

TEST REPORT

Test requested: ***In vitro* cytotoxicity test (as per ISO 10993-5)**
 Test on extract

Test material:

Number of test materials: 2
 Nature of test material: Plastic
 Test material code: TM1
 TM2

Study dates:

Test material received: 18 July, 2016
 Begin of testing: 19 July, 2016
 End of testing: 21 July, 2016
 Final report: 21 July, 2016

Cell line: L929 mouse fibroblast NCTC clone 929 strain L

Controls:

Negative control: Cell culture medium incubated under the same conditions as the extracts
 Positive control: Dilution series of Dimethylsulfoxide

Test method:

Preparation of Extracts and controls: Extracts, negative and positive controls were prepared in accordance with ISO 10993-12.

Test procedure: The test was carried out as per ISO 10993-5.

Results:

Table 1- Qualitative morphological grading of cytotoxicity of extracts

	Score values as defined below				
	Concentration of dilutions (v/v)				
	100%	30%	10%	3%	1%
TM 1	0	0	0	-	-
TM 2	1-2	1	0	-	-
Negative control	0	-	-	-	-
Positive control	-	-	4	2-3	0

Definition of scores

0 = No reactivity Discrete intracytoplasmic granules, no cell lysis, no reduction of cell growth
 1 = Slight reactivity Not more than 20% of the cells are round, loosely attached and without intracytoplasmic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
 2 = Mild reactivity Not more than 50% of the cells are round, devoid of intracytoplasmic

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	granules, no extensive cell lysis; not more than 50 % growth inhibition observable.
3 = Moderate reactivity	Not more than 70% of the cell layers contain rounded cells or are lysed; cell layers not completely destroyed, but more than 50 % growth inhibition observable.
4 = Severe reactivity	Nearly complete or complete destruction of the cell layers.

Table 2- Quantitative measurements of cytotoxic effects by MTT assay

	Mean value of optical density at 570nm					Viability %				
	Concentration of dilutions (v/v)					Concentration of dilutions (v/v)				
	100%	30%	10%	3%	1%	100%	30%	10%	3%	1%
TM 1	0.765	0.767	0.784	-	-	97	97	100	-	-
TM 2	0.587	0.688	0.689	-	-	67	84	86	-	-
Negative control	0.786	-	-	-	-	100	-	-	-	-
Positive control	-	-	0.219	0.553	0.783	-	-	7	61	100
Blank	0.179	-	-	-	-	-	-	-	-	-

$$\text{Viability \%} = 100 \times \text{OD}_{570t} / \text{OD}_{570c}$$

where

OD_{570t} is the mean value of measured optical density of the test material after subtracting blank (medium control)

OD_{570c} is the mean value of measured optical density of the negative control after subtracting blank (medium control)

References:

- ISO 10993-5, 2009, Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity
- ISO 10993-1, 2003, Biological evaluation of medical devices - Part 1: Evaluation and testing
- ISO 10993-12, 2008, Biological evaluation of medical devices - Part 12: Sample preparation and reference materials