

**TEST REPORT no. E-039-PB-17**

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Test laboratory accredited by Deutsche Akkreditierungsstelle GmbH (DAkkS) against DIN EN ISO/IEC 17025. The accreditation is valid for the testing procedures listed in the DAkkS-certificate (in the test report signed with \* or \*\*).



Member of **eurolab**-Deutschland  
Verein deutscher Prüflaboratorien e.V.

**Customer:**

Innofa B.V.  
Minosstraat 20  
NL-5048 CK Tilburg

**Order data:**

Order date: 21.02.2017  
Samples received: 21.02.2017  
Testing date: 22.02.2017 –  
02.03.2017  
Your reference: -

**Task:**

Testing of cytotoxicity, biological evaluation of medical devices - Part 5:  
Testing for in vitro cytotoxicity (ISO 10993-5: 2009)

**Received Sample(s):**

Sample name	Sample specification/ description
1)	P114 – 4001 – 024 – 05 11 k 000 batch: E 2111 29001 2

**1 Test methods/ normative references applied**

Biological evaluation of medical devices- Part 5: Test for in vitro cytotoxicity according to DIN EN ISO 10993-5:2009-10 \*\*, testing with MTT

## **2 Sampling and tests carried out**

### **Test principle:**

The MTT assay is a quantitative colorimetric method to determine cell proliferation and viability.

The MTT assay utilizes yellow colored tetrazolium salt MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide) which is metabolized by mitochondrial succinic dehydrogenase activity of viable cells to purple formazan reaction product. After dissolving, formazan is quantified photometrically.

Toxic agents lead to cell damages including mitochondrial dysfunction. This is indicated by less formazan reaction products and lower color intensity (lower absorbance).

### **Sampling, sterilization and preparation:**

Sampling was done by the customer. The samples were gammasterilized [25 kGy]

Extraction was carried out for 24 hours at a temperature of 37 °C. Extraction ratio was 0,1 g/ml; culture medium including serum (10 % FBS) was used as extraction medium.

L-929 mouse fibroblast cells were seeded on multiwell plates in cell culture medium, each of 10.000 cells per well. 24 hours after seeding test extract was added to adherent cells in concentrations of 10 %, 20 %, 50% and 100 %. After an incubation period of 24 hours, supernatant was decanted and MTT solution was added. Purple colored formazan reaction product was dissolved in isopropyl alcohol and quantified photometrically by measuring absorbance.

3 extracts were prepared from the sample. Every extract was tested in quadruplicate. As a positive control formaldehyde was used. Phosphate buffer was used as a negative control. As reference value (100 %-value) test was done without sample ("control").

Sample is evaluated non cytotoxic if absorbance values –considering 100 % extract concentration- were > 70% in relation to reference value.

### **3 Test results**

The detailed results are shown graphically in the annex.

The tested sample did meet the requirements described and are considered **non cytotoxic** according to standard ISO 10993-5.

### **4 Discussion of results**

None

Denkendorf, 07.03.2017

Nicole Müschenborn  
(Head of Laboratory)

Alexander Ott  
(Laboratory Assistant)

### **Annex Test data**

**Note:**

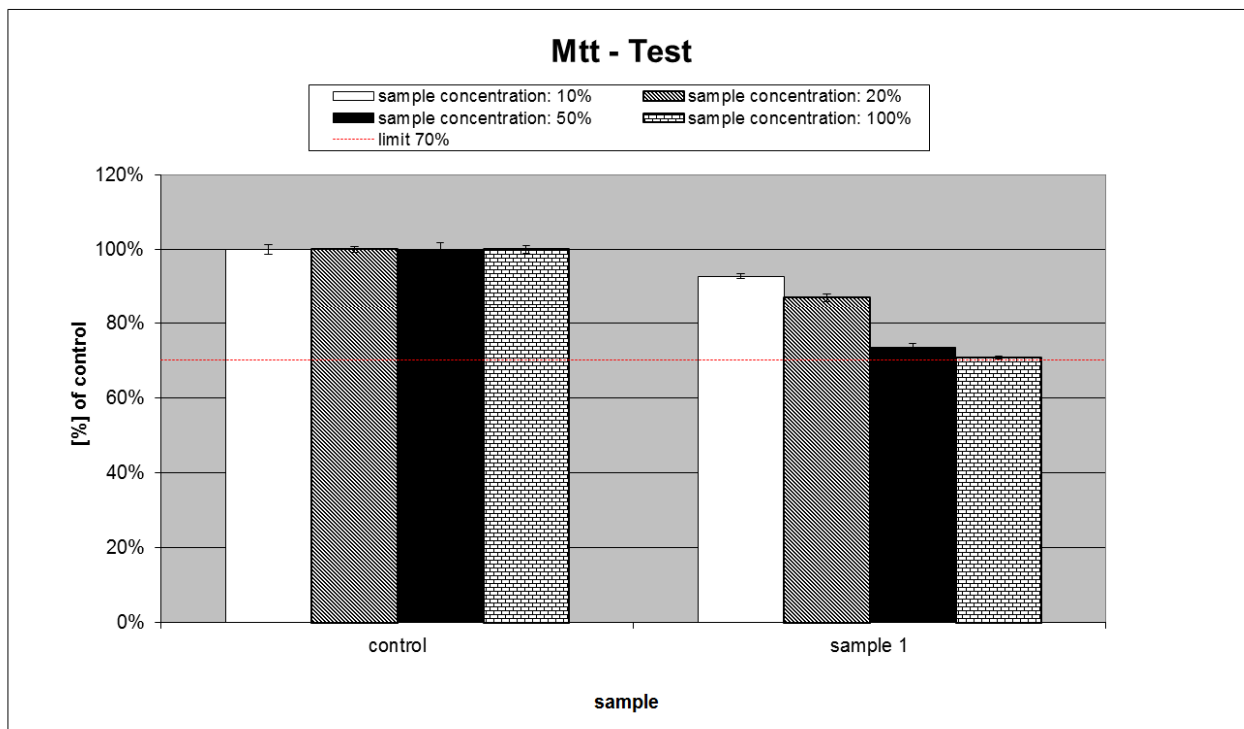
The test results relate to the above-specified samples, and may no be used as a basis for a lawsuit or advertisement without any written permission by the testing laboratory. The test report shall not be duplicated in parts without written permission of the testing laboratory. Evaluations of the obtained results and conclusions do not form part of the accreditation.

Retained samples will be discarded after 2 years, documents and data after 10 years without consultation, unless otherwise agreed.

sample:

test:	MTT-Test (Nr.: 1093)	test order:	E039PB17	operator:	Ott
cells:	L-929	cell number:	10.000	culture vessels:	96-multiwell
preincub.:	24 h	incubation with samples:	24 h	date:	28.02.2017
sterilization-method:	Gamma-sterilisation (25 kGy)	media:	MEM (Co. Biochrom) stab. Gln; Lot.: 0703 E + 10% Fbs Superior (Co. Biochrom) Lot.: 0959 E		
extraction conditions:	24 h at 37°C in Media with 10 % Fbs; a) = 0,4107 g / 4,107 ml; b) = 0,4154 g / 4,154 ml; c) = 0,4621 g / 4,621 ml; PK = je 1 x Givf (Dampst.) + 100µl SDS (2500 µg/ml; stf.) 5 Tage getrocknet in 5 ml				

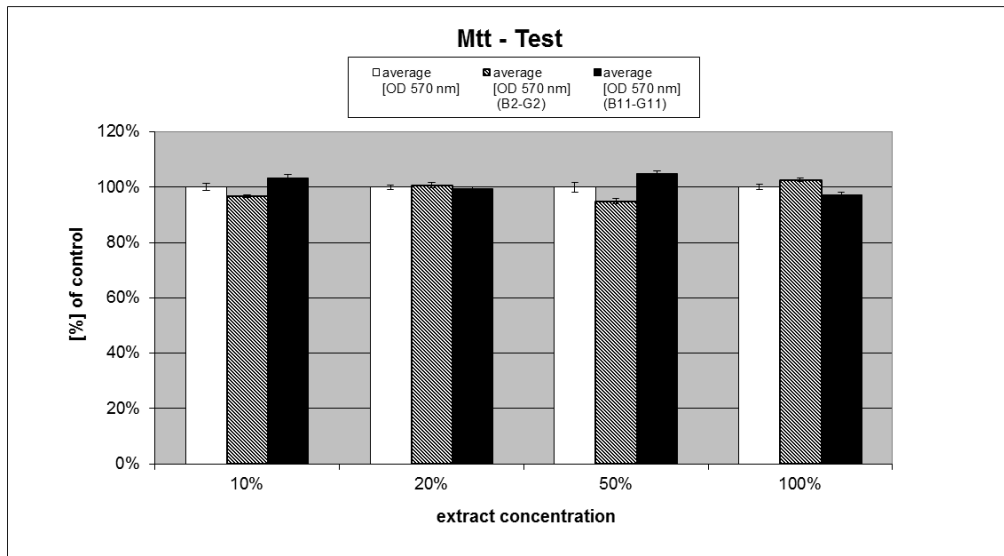
sample	conc.: [%]	average [OD 570 nm]	st. deviation	% of control	conc.: [%]	average [OD 570 nm]	st. deviation	% of control
control	10	0,259	0,013	100%	20	0,266	0,008	100%
sample 1	10	0,240	0,007	93%	20	0,232	0,011	87%
control	50	0,288	0,017	100%	100	0,250	0,010	100%
sample 1	50	0,212	0,011	73%	100	0,177	0,005	71%



negative sample:

<b>test:</b>	MTT-Test [Nr.: 1093]	<b>test order</b>	E039PB17	<b>operator</b>	Ott
<b>cells:</b>	L-929	<b>cell number:</b>	10000	<b>culture vessels:</b>	96-multiwell
<b>preincub.:</b>	24 h	<b>incubation with samples:</b>	24 h	<b>date:</b>	28.02.2017
<b>media:</b>	MEM (Co. Biochrom) stab. Glü; Lot.: 0703 E + 10% Fbs Superior (Co. Biochrom) Lot.: 0959 E				

extract concentration of controls	average [OD 570 nm]	st. deviation	% of control	average [OD 570 nm] (B2-G2)	st. deviation	% of control	average [OD 570 nm] (B11-G11)	st. deviation	% of control
10%	0,259	0,013	100%	0,250	0,004	97%	0,267	0,013	103%
20%	0,266	0,008	100%	0,268	0,009	101%	0,264	0,008	99%
50%	0,288	0,017	100%	0,274	0,009	95%	0,303	0,009	105%
100%	0,250	0,010	100%	0,256	0,006	103%	0,243	0,007	97%



positive sample:

test:	MTT-Test (Nr.: 1093)	test order	E039PB17	operator	Ott
cells:	L-929	cell number:	10000	culture vessels:	96-multiwell
preincub.:	24 h	incubation with samples:	24 h	date:	28.02.2017
media:	MEM (Co. Biochrom) stab. Gln; Lot.: 0703 E + 10% Fbs Superior (Co. Biochrom) Lot.: 0959 E				

samples	conc.: [%]	average [OD 570 nm]	st. deviation	% of control	conc.: [%]	average [OD 570 nm]	st. deviation	% of control
Pbs	10	0,252	0,009	100%	20	0,249	0,005	100%
F30	10	0,132	0,011	52%	20	0,116	0,004	46%
F50	10	0,100	0,008	40%	20	0,078	0,004	31%
T50	10	0,220	0,009	87%	20	0,174	0,006	70%
T75	10	0,188	0,003	75%	20	0,147	0,006	59%

