Test report

Skin Irritation Test

Test report Number: ISO 201508-01742_SI_engl

Product distributed and saled by:

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Sep 11th 15

Test material:

PU-Film for Breast Prosthesis

Test material received: Aug 17th 15 Test performed: Sep 04th 15

Result	**The PU-Film for Breast Prosthesis didn´t cause a skin irritating effect.**

Description of the test procedure:

Normative References: ISO 10993-10 (2009), ISO 10993-1 (2009), OECD TG 439

Based on the normative references ISO-10993-1 (2009), Chapter 4.6 and ISO 10993–10 (2009) an *in vitro* test was performed to evaluate the potential skin irritating effect of the test material. An *in vitro* reconstituted, human epidermal 3D-skin model, type epiCS, Lot. Nr. 100-AE1608-1 was used. The test was performed according to ECVAM´s "Performance standards for applying human skin models to *in vitro* skin irritation testing".
Prior to performing the test procedure the skin model cell culture inserts were incubated for 24 h at 37°C and 5 % pCO_2 in a cell culture incubator in fresh Maintenance Medium.
After this preincubation time 50 µl of PBS was pipetted on the surface of each skin model. Then the material samples to be tested were applied on the skin surface using a pair of sterile tweezers.

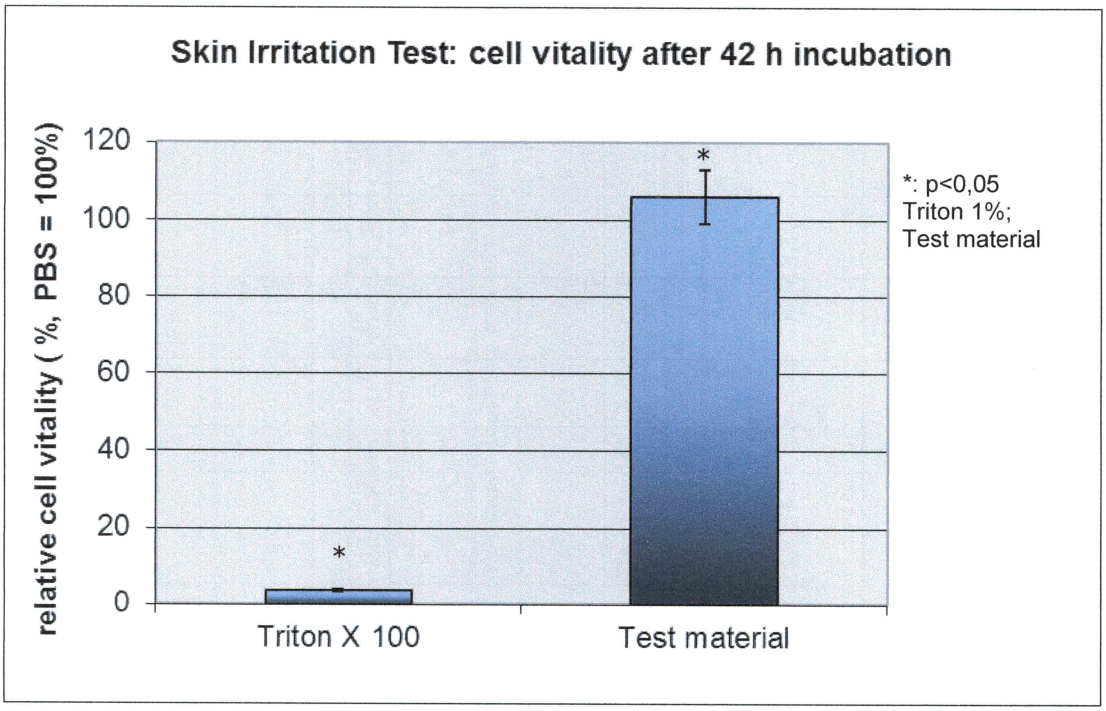
50 µl Triton X 100 were used as a (skin irritating) positive control, 50 µl PBS were used as a (not skin irritating) negative control. All experiments were performed in duplicate. After 20 min incubation all inserts were rinsed thoroughly with sterile PBS, blotted on a paper towel to remove excess PBS and cultivated in Maintenance Medium for 42 h in a cell culture incubator at 37°C and 5 % pCO_2 .

Measurement of LDH-release:
After the 42 h incubation period 2 x 100 µl Maintenance Medium Samples were taken from every skin model and the LDH-release was measured using a „Cytotoxicity Detection Kit (LDH)" from Roche Diagnostics.

Measurement of cell vitality (MTT-test):
The inserts were rinsed once in Assay Medium using a 24-well cell culture plate and transferred in a second 24-well cell culture plate with 300 µl Cellsystems Assay Medium containing 1mg/ml MTT (Sigma M5655). All inserts were incubated for additional 3 h in a cell culture incubator at 37°C and 5 % pCO_2. Afterwards all inserts were blotted on a paper towel and the MTT-dye was extracted from the skin samples using 2 ml Isopropanol per insert. The extinction of each Isopropanol extract was measured in a photometer at 570 nm. With this data the relative vitality of the cells in the skin samples compared to the negative control (PBS) was calculated.

Results:

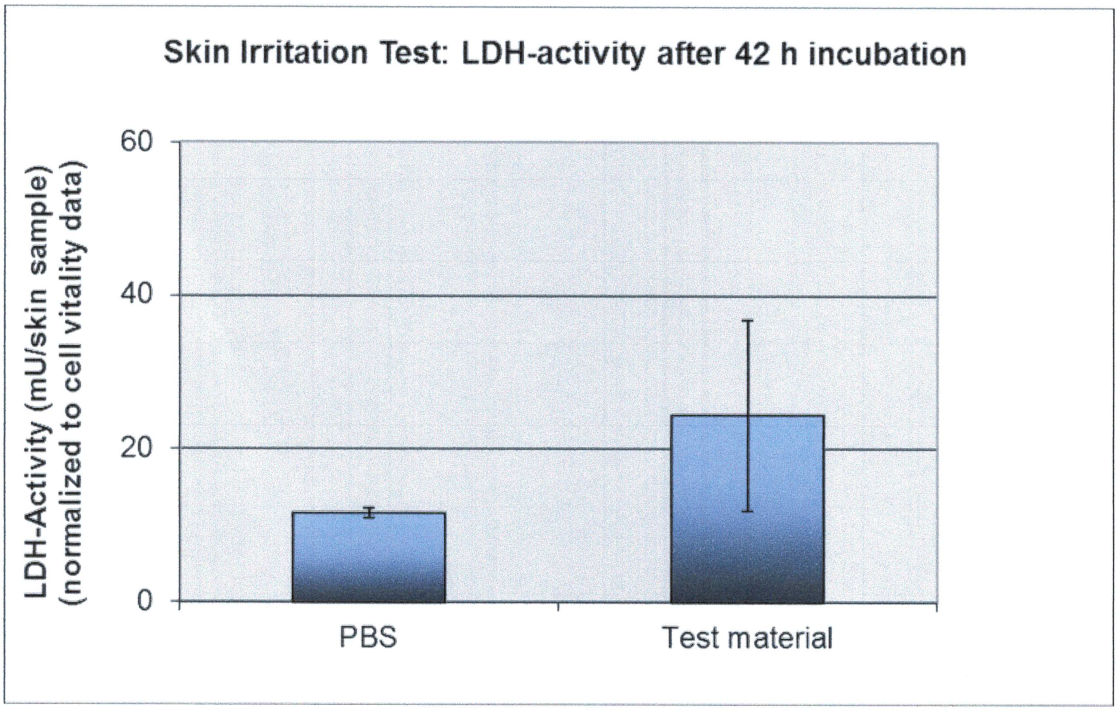
Measurement of cell vitality (MTT-test)



Result data	n=2, (%), (PBS data not shown in graphic)		
(rel. cell vitality)	Triton X 100	PBS	Test material
Mean	3,71	100	106,14
Standarddev.	0,39	7,65	6,90

In the presence of Triton X 100 on the skin culture inserts 2,7 % of the cell vitality compared to the negative control was reached. This value is within the valid range of 15 % cell vitality or less compared to the negative control.
A material is considered as skin irritating, if it reduces the cell vitality of the skin samples to less than 50 % compared to negative control skin samples. This is not the case in this experiment. The material didn´t show a skin irritating effect.

LDH-Release



LDH-Release (mU/skin sample)	Triton X 100 data not shown in graphic, n=2		
	Triton X 100	PBS	Test material
Mean	1805,04	11,60	24,46
Standarddev.	538,51	0,64	12,42

Skin samples charged with the test material didn´t increase the LDH-Release significantly compared to the PBS negative controls.

Result	**The PU-Film for Breast Prosthesis didn´t cause a skin irritating effect.**

Explanatory notes:

none

Test performed by: Dietmar Scheddin

authorized by: Dietmar Scheddin
(Dr. D. Scheddin / CEO CYTOX)

DECLARATION

Description: Polyurethane Breast Prostheses

To Whom it May Concern;

Trulife hereby declares that the aforementioned products are suitable for use in sea water and chlorine, however these are not tested in this environment and may experience some defects after prolonged use if not cleaned appropriately.

Signed: _____ Date: 28/06/2023

Rebecca Holmes
Senior Quality Specialist