Test report

Cytotoxicity test according to ISO 10993-5 (2009)

Test report Number: ISO 201212_01230_3P_engl

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Mar 05th 13

Test material:

Oxygen, produced by the Oxygen Generator „OXYFLY"
(device configuration as of Feb 2013)

Test material received: Feb 14th 13 Test performed: Feb 24th 13

Result	**The oxygen, produced by the Oxygen Generator „OXYFLY" (device configuration as of Feb 2013) didn´t cause a cytotoxic effect.**

Description of the test procedure:

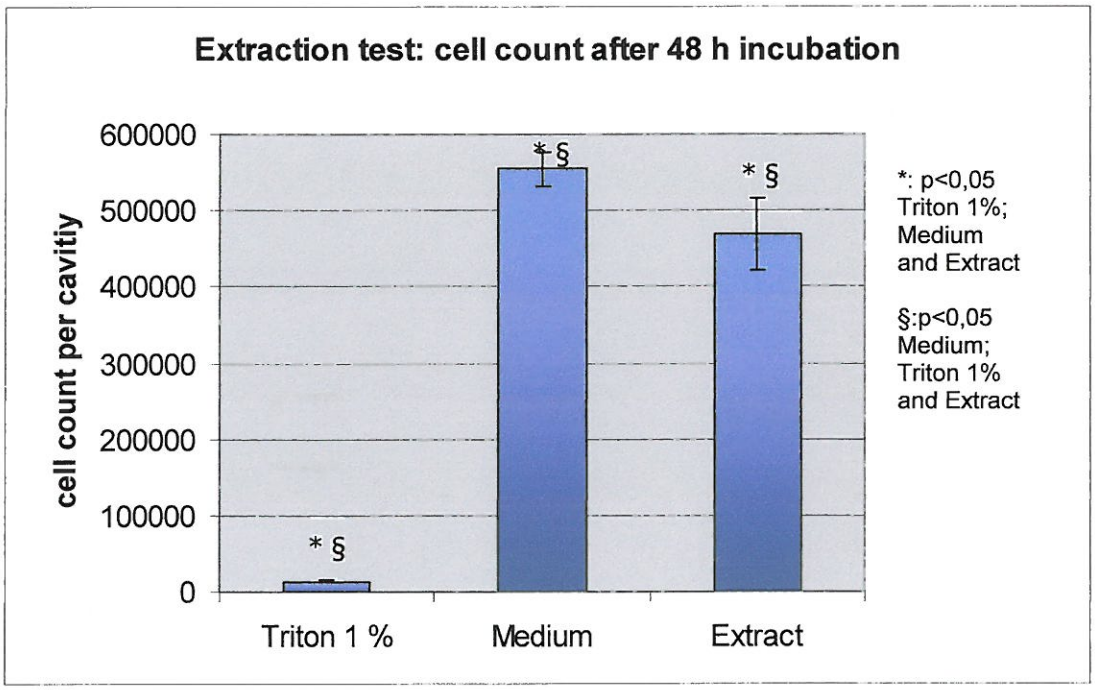
Normative Reference: ISO 10993-5 (2009)

The Oxygen produced under normal load by the Oxygen Generator „OXYFLY" was blown in for 15 min in 30 ml extraction medium (DMEM with antibiotics but without FCS).
After completion of the blow-in procedure the extraction medium was sterile filtered and supplemented with sterile FCS (concentration: 10 % FCS in extraction medium). The FCS-supplemented extraction medium was pipetted under sterile conditions on precultivated L929 mouse fibroblast cells (initial seeding cell concentration 50.000 cells/ml) and incubated for 48 h at 37°C and a partial pressure of 5 % pCO_2.
Triton X 100 was used as a toxic control substance (concentration in the experiment: 1 % v/v). Cell culture medium was used as a non-toxic control.
After the 48 h incubation period the release of Lactatdehydrogenase (LDH) in the cell culture supernatant was measured using a photometric procedure. The cell were lysed

using an alkaline lysis procedure and the protein content was determined by the method according to Bradford.
In a second 24-well cell culture plate the cells were fixed and stained with methylene blue for the determination of the cell count. After acid extraction of the methylene blue the dye concentration was measured using a photometric procedure. Extinction data was compared to a standard curve to determine the cell count by the extinction of this cell specific dye.

Results:

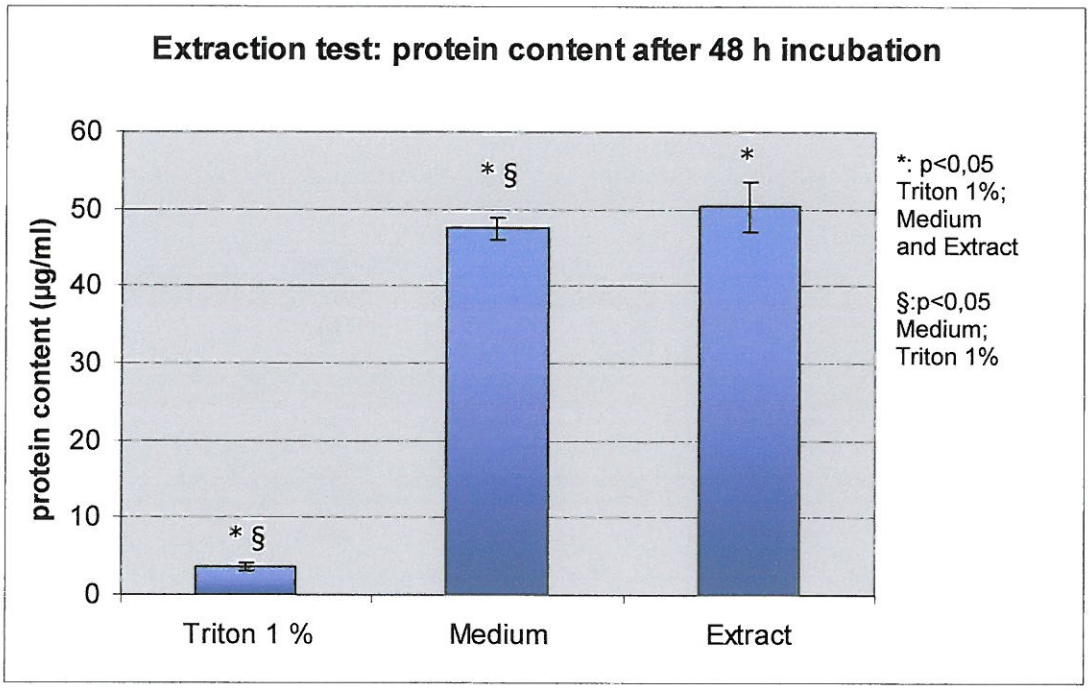
Cell count



Result data	Cell count, n=4		
	Triton 1 %	Medium	Extract
Mean	13834	554391	469246
Standarddev.	972,15	21357,66	46463,87

In the presence of Triton X 100 in the cell culture medium 2,5 % of the cell count compared to the negative control was reached. This value is within the valid range of 15 % cell count or less compared to the negative control.
An extract is considered cytotoxic, if an extract leads to a cell count of the test cells of less than 70 % compared to the negative control. This is not the case in this experiment. The extract didn´t show a cytotoxic effect.

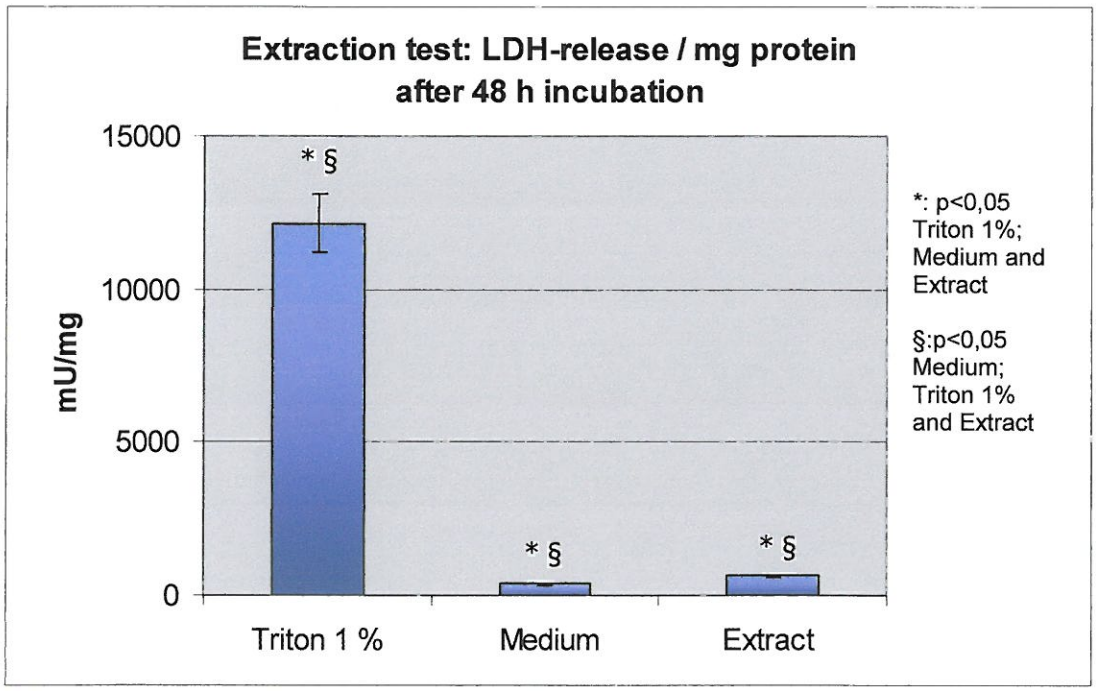
Protein content



Result Data µg/ml	Protein content, n=4		
	Triton 1 %	Medium	Extract
Mean	3,61	47,49	50,30
Standarddev.	0,51	1,40	3,14

In the presence of Triton X 100 in the cell culture medium 7,6 % of the protein content compared to the negative control was reached. This value is within the valid range of 15 % cell count or less compared to the negative control. An extract is considered cytotoxic, if an extract leads to a protein content of the test cells of less than 70 % compared to the negative control. This is not the case in this experiment. The extract didn´t show a cytotoxic effect.

LDH-release



Result Data	LDH-release, n=4		
mU/mg	Triton 1 %	Medium	Extract
Mean	12160,39	366,34	648,87
Standarddev.	920,04	42,21	34,10

In the presence of Triton X 100 in the culture medium the specific LDH-activity is more than 33 times higher compared to the negative control.

Result	**The oxygen, produced by the Oxygen Generator „OXYFLY" (device configuration as of Feb 2013) didn´t cause a cytotoxic effect.**

Explanatory notes:

none

Test performed by: _____

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