

TRANSLATION

TÜV Product Service GmbH
Masurenweg 1 – 3

30163 Hannover

**Toxicity examination according to DIN EN ISO 10993-5
SOP 09-001**

Test Report

Sample no.:	SN 22001
Date of delivery:	10.09.2001
Product:	Skai sample black no. 5016/9 Skai sample grey no. 5016/5
Client:	TÜV Product Service GmbH
Manufacturer:	INVACARE Deutschland GmbH
Test method:	Toxicity of eluates in accordance with DIN EN ISO 10993-5, 1999-11 Biological assessment of medical products Part 5: Testing toxicity SOP 09-001
Test period:	10.09.2001 – 14.09.2001
Test conditions:	Climate: 22°C/25 % rel. Humidity Conditioning: 24 hours

Description of the test method

Conditions of extraction: 5 cm² material (approx. 1 g) in 4 ml MEM + 1 % serum + 1 % antibiotic solution at 37°C for 24 h = extraction medium

Methodic: Cell cultivation
FL-cells are a cell line of the human amnion. They are conserved in 250 ml-bottles (Greiner GmbH). All cells are filtered in 4 d. After the 100th filtration the reserve has been regenerated from the standard culture.
For test purposes the cells are put into culture tubes after having been separated.
The cultivation medium consists of 70 % lactalbuminhydrolysate and 30 % MEM with additives of 1 % antibiotic solution (final concentration: 100 IE Penicillin G and 100 µg streptomycin-sulfat/ml) and 8 % bovine serum.
The p_H-value was adjusted to 7,2 with M NaOH solution.

Exposition: After 72 hours cultivation the cells presented as monolayer, and a transfer to the incubation substrate was made. This consists of MEM + 1 % antibiotic solution + 1 % bovine serum (control), or above mentioned extraction medium directly has been used, respectively. For that purpose the medium was taken out and carefully replaced by the fresh medium (control or extraction medium), (1,5 ml per tube). A 24-hours incubation follows.

Removing/counting the cells: The medium was taken out, the cells were made wet equally by 0,3 ml enzym solution (0,05 % 1:250 trypsin and 0,02 % EDTA in Ca²⁺ and Mg²⁺ and free PBS) and the solution was taken off. After approx. 10 min incubation of the tubes at 37° the separated cells are suspended in each 1 ml MEM and 1 % serum, and the amount of cells comes out by means of the Universal-counter.

Analysis: The cell figure of 19 test samples has been determined and finally statistically evaluated.

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