Cytotoxicity test report

Customer name:
Azar Teb Asoudeh Company
Address: Basement, No. 1, Farsad Hemmati alley, Before Sajjadieh Bridge, Niayesh Highway, Tabriz, Iran
Phone number: +98-914-4010871, E-mail: sales@asoudehbiotech.com, web: asoudehbiotech.com

Test place:
KiaNonoBioVista laboratory
Address: Unit 10, Floor 4, No. 58, Rose building, Taherian Street, Sadeghiyeh Square, Tehran, Iran
Phone number: +98-21-440240223, E-mail: info@kiananobio.com, web: kiananobio.com

Reference Standard:
- ISO 10993-5:2009 "Biological evaluation of medical devices - Part 5: Test for in vitro cytotoxicity"

Time Schedule:

Sample receiving date:	04/01/2022
Start test date:	04/01/2022
End test date:	11/01/2022

Sample Identification:

Name:	Conical tube with cap sterile
Manufacturer:	Azar Teb Asoudeh Company - Iran
Sample code in lab.:	S001012/677/06
Manufacturing Date:	09/2019
Expiry date:	09/2022
LOT Number:	ASC10TKP1
REF Number:	-
Batch Number:	-
Sterilization method:	R

Test Summary Report:

Test type	Test method	Test results	Issue date
In vitro cytotoxicity: Test on sample extract	ISO 10993-5:2009 Annex C (MTT)	Non-Cytotoxic	12/01/2022

Sajjad Mohammadi
KiaNanoBioVista Lab Manager

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ثبت ۵۰۳۲۱۵ سهامی خاص

Test and conformity methods:

- ISO 10993-5:2009 Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity
- ISO 10993-12:2012 Biological evaluation of medical devices - Part 12: Sample preparation and reference materials

Test Materials:

Fetal bovine serum (FBS), Roswell Park Memorial Institute (RPMI), Phosphate buffered saline (PBS), Trypsin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid (MTT), Dimethyl sulfoxide (DMSO), High Density Polyethylene (HDPE), Latex from glove, Mouse fibroblast cell line (L929), Streptomycin and penicillin.

Equipment:

Laminar flow hood (Jal Tajhiz)
CO_2 incubator (Memmert)
Inverted microscope (Labomed)
Photometer microplate reader (BioTek)
Centrifuge (Hettich)

Test Method:

Sample Preparation

Under laminar flow hood in aseptic condition samples were filled with extraction solution (RPMI culture medium). The sample extract was obtained by overfilling the test sample in RPMI to reach a surface/volume ratio of 3cm²/ml. The extract of the negative and positive control were prepared by immersing HDPE in RPMI, in order to reach a surface/volume ratio of 3cm²/ml and immersing latex in RPMI, in order to reach a surface/volume ratio of 6cm²/ml respectively. Then all samples were incubated at 37°C±1°C for 72±2 hours in dynamic condition. In particular, extraction solutions were used immediately after preparation. No abnormalities were observed in extract appearance: no signs of particles, clouding, discoloring or chemical precipitation were recorded, the extract were not filtered before use.

Experimental Design

The test has been carried out using the cellular line L929 that has a high capacity to proliferate (as recommended in ISO 10993-5). Cell cultures were grown until a confluent monolayer in flask and then a cell suspension (1×10^4) in RPMI culture medium containing 10% fetal bovine serum (FBS), 1% Streptomycin and penicillin was seeded inside 96-wells plates (with 6 repetitions). The plate was placed in incubator containing 5%CO_2 atmosphere at 37±1°C. After 24±2 hours when the cells are completely attached to the bottom of plate, the culture medium was replaced with the samples extract and incubated at 37 °C±1 for 24±2 hours. At the end of incubation, the extracts were removed and MTT solution (0.5 mg/ml) was added to each well. After 3 hours, MTT solution was replaced with DMSO to generate purple. Negative control and positive control were prepared at the same time and submitted to the same process of the sample. The color intensity was measured using ELISA reader at 570 nm wavelength. The cell viability rate (%) was calculated in the following equation (the wells containing more living cells demonstrate higher optical density):

$$\text{Viability\%} = \frac{\text{mean of OD sample}}{\text{mean of OD control}} \times 100$$

Acceptance criteria:

Mean OD_{570} of negative controls must be ≥0.2.
Standard deviation of the same sample must be ≤15%.
Viability of positive control must be ≤50%.
Sample has a cytotoxic potential if cellular viability is reduced to <70%.
Sample has a non-cytotoxic potential if cellular viability is reduced to ≥70%.

Results:

	Sample extract	Blank	Positive control	Negative control
Average (OD)	0.488	0.494	0.008	0.490
Viability %	**98.825**	100	1.621	99.271
STDEV	0.01	0.01	0.02	0.03

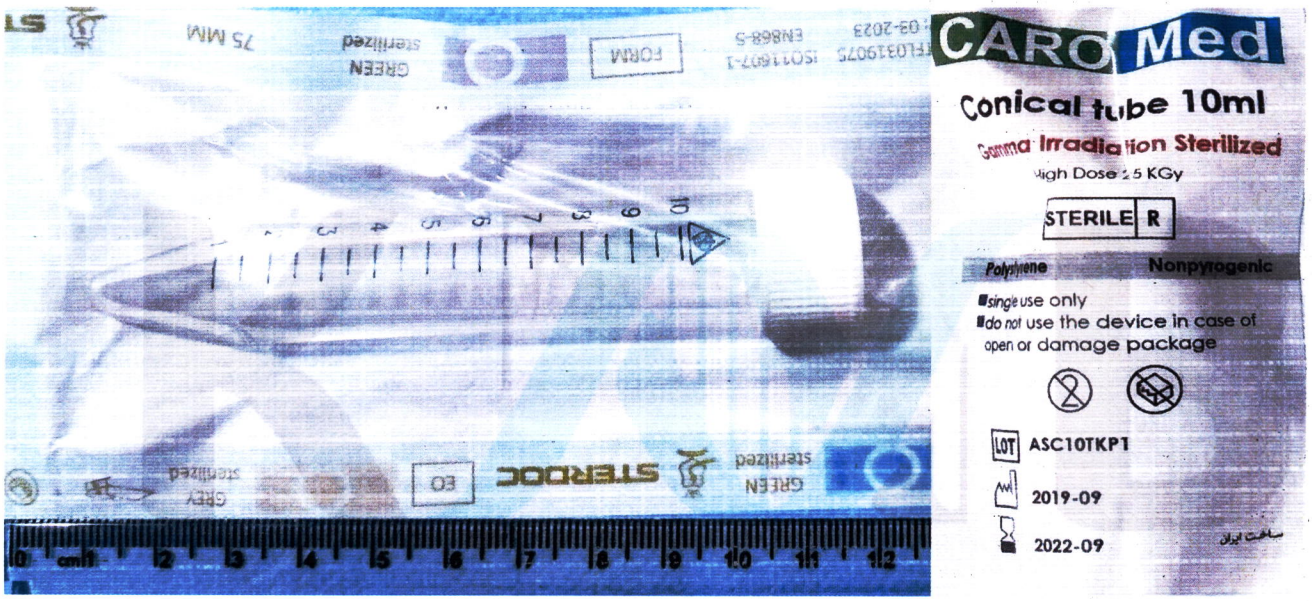
Expanded uncertainty=±4.34 % Reading *Expanded uncertainty: An interval about the measurement result that encompasses 95% of probability density function of measurement result.

Reporting statements of conformity:
On the basis of the results, interpreted according to ISO 10993-5:2009 the test sample `` Conical tube with cap sterile - Azar Teb Asoudeh Company `` is considered **NON-CYTOTOXIC**.

Interoperation of results:
Not applicable

Record filing:
The study program and all raw data will be retained in Kiananobiovista Lab archives for a period of 10 years from the issue of the final report. An original copy of the report is available at Kiananobiovista Lab.

Sample test image:



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