PRODUCT SPECIFICATION

instaCELL Cytotoxicity Assay Kit

CatN°: SF020-01
Lot#: CX-24022021

PRODUCT DEFINITION

Test kit to assess the cytotoxicity of chemicals and leachables by their application to cultures of mammalian cells and the subsequent determination of cell viability.

QUALITY SPECIFICATION OF THE CELLS

	Batch Quality Control	Specification Limits
Cell Count[1]	**1.04E+07**	≥ 90% of nominal cell count
Homogenity (cell count)	**98%**	≥ 90%
Viability (after thawing)[1]	**97 %**	≥ 90 %
Proliferative Capacity[5]	**100%**	≥ 70%
Debris/Cell Ratio[1]	**0.2**	≤ 1.0
Aggregation[1]	**1.2**	≤ 2.0
Sterility (bacteria, yeast, fungi)[2]	**passed**	negative after 4 days
Sterility (mycoplasma)[3]	**passed**	negative by PCR
Morphology	**passed**	unalterd to refference
Cytotoxicity Assay (IC50 reference substances)[4]	**A: 0.88 M** **B: 9.35E-3 M** **C: 1.40E-5 M**	A: 6.1E-01 M < x < 1.2E-00 M B: 9.8E-04 M < x < 2.7E-02 M C: 1.4E-06 M < x < 4.0E-05 M
Cytotoxicity Assay (Z')[4]	**0.92**	> 0.5

[1]: as determined by CASY TT cytometer, [2]: by microscopic/visual control after four days of culture in the absence of antibiotics, [3]: as determined in the cell culture supernatant after four days of cultures by PCR, [4]: dose response of three reference compounds (A: Glycerol, B: Antipyrine, C: Sodium Selenite) performed according to the assay protocol. [5] Proliferative Capacity shows the percentage of cell activity 72 hours after seeding in relation to cells from continuous culture (100%). Both types were seeded with the same cell density at t=0.

KIT CONTENT

	Lot#
Recovery Buffer A	**91-210125NR01**
Assay Buffer A	**91-210113NR01**
Assay Medium A	**91-210107NR02**
Cytotoxic Control	**91-210107NR03**
Resazurin Solution	**91-210121NR01**
96-well Assay Plate	**I184522M**
Assay Ready L-929 Cells	**92-200709JP01**

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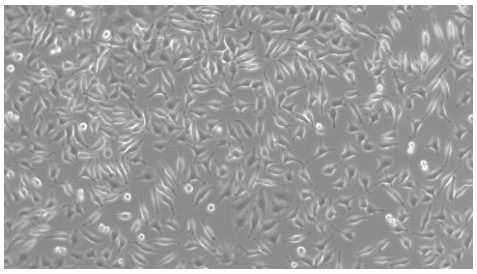
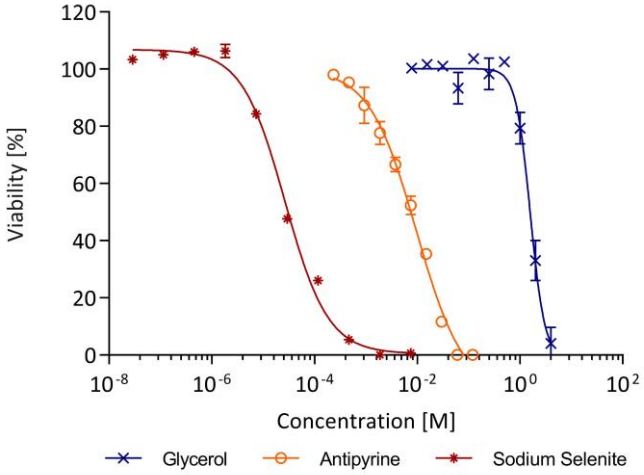
MORPHOLOGY



Morphology of cells 24 hours after seeding

CYTOTOXICITY ASSAY



Dose response of three reference compounds (A: Glycerol, B: Antipyrine, C: Sodium Selenite) performed according to the assay protocol.

METHODS

Cell Viability Parameters: Viability parameters (viable cell count after thawing, grade of aggregation, percentage of debris) were determined from a pooled sample (SOP-2015-02). Briefly, assay ready cells were thawed in a water bath. 50 µl of each sample were pooled, diluted 1:1000 in CASY Ton buffer and measured (3 replica) in a CASY TT automatic cell counter. Vial to vial variation was determined in a plate-based viability assay. Equal aliquots of each cell samples were dispensed into a 96 well-plate. A cellular dye was added to the well to obtain fluorescent signal which is proportional to the viable cell count.

Proliferative Capacity: 1,0E+04 cells from the assay ready cell samples and from a reference of a continuously passaged culture were seed in a 96-well plate (8 replica each). After 48 hours of cultivation the proliferation of the cells was determined by addition of a metabolic cell dye (Resazurin). The proliferative capacity of the assay ready cells is calculated in percent of the exponentially growing reference. (SOP-2017-03).

Sterility Testing: Assay ready cells were seed in two specific bacteria growth brothes (Tryptic Soy Broth for aerob and Thioglycollate broth for anaerob conditions) and cultivated over a course of 14 days. After day 1, 4, 7 and 14 the cultures were analyzed microscopically for cell growth, cell morphology, and incidences of contamination (bacteria, yeast, or fungi). For mycoplasma testing from a three days old, sub-confluent culture 500 µl of the supernatant was taken and analyzed by PCR using a Mycoplasma detection kit (Minerva). Assay was performed according to the manufacturer protocol (SOP-2015-06).

LIMITED USE

The product is provided under the terms of a limited use license provided with kit. By breaking the sealed bag, the user is explicitly accepting the terms for limited use.

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