PRODUCT SPECIFICATION

instaCELL Cytotoxicity Assay Kit

CatN°: SF020-01
Lot#: CX-18032021
Expiry Date: 09.07.2022

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

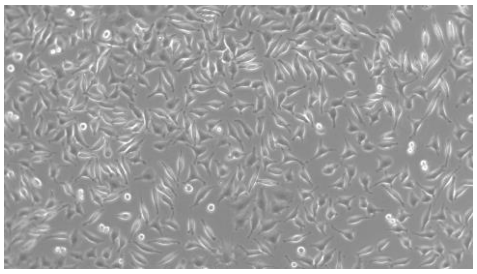
	Batch Quality Control	Specification Limits
Cell Count	**1.04E+07**	9.00E+06<>1.20E+07
Homogenity (cell count)	**98%**	≥ 90%
Viability (after thawing)	**97 %**	≥ 90%
Proliferative Capacity	**100%**	≥ 70%
Debris/Cell Ratio	**0.2**	≤ 1.0
Aggregation	**1.2**	≤ 2.0
Sterility (bacteria, yeast, fungi)	**passed**	negative after 7 days
Sterility (mycoplasma)	**passed**	negative by PCR
Morphology	**passed**	unalterd to reference
Cytotoxicity Assay (IC50)	**Glycerol : 1.8 M** **Antipyrine : 1.2E-3 M** **Sodium Selsnite : 6.6E-5 M**	1.0E+00 M < x < 2.0E+00 M 4.0E-03 M < x < 4.0E-02 M 1.0E-05 M < x < 2.0E-04 M
Cytotoxicity Assay (Z')	**0.94**	> 0.5

KIT CONTENT

	Lot#	Storage Temperature
Recovery Buffer A	**91-210316NR02**	**-20°C**
Assay Buffer A	**91-210316NR01**	**-20°C**
Assay Medium A	**91-210316NR03**	**-20°C**
Cytotoxic Control	**91-210317NR04**	**-20°C**
Resazurin Solution	**91-210317NR02**	**-20°C**
96-well Assay Plate	**I184522M**	**Room Temperature**
Assay Ready L-929 Cells	**92-200709JP01**	**< -140°C**

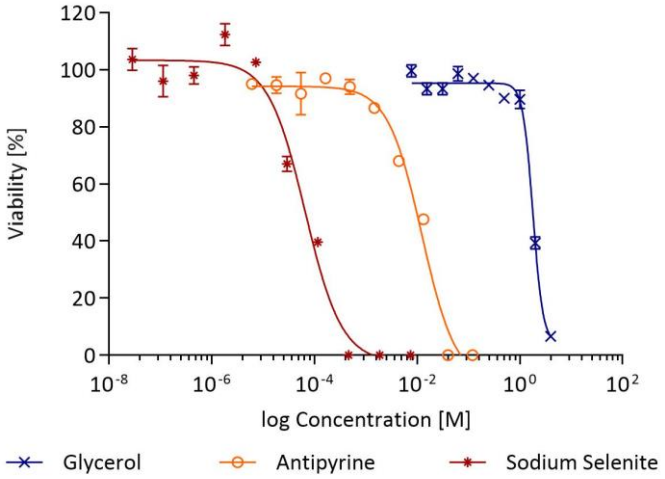
Sterility was analyzed by microscopic/visual control after seven days according to sterility testing. Functionality of the content was tested by performing the assay with all listed batches.

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. 100 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.

Proliferative Capacity: Proliferative Capacity compares mean growth rate (T0 - T72 hours) of all sample vials with mean growth rate of exponentially growing culture. Freshly thawed cells from the assay ready cell samples were seed in a 96-well plate (3 replica each) according their specific 3 day seeding density. After 72 hours of cultivation, the proliferation of the cells was determined by addition of a metabolic cell dye (Resazurin) (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).

Cytotoxicity Assay: The cells were seeded at 7E+04 c/well in an 96-well plate and treated with the reference substances Glycerol, Antipyrine and Sodium Selenite for 24h at 37°C and 5% CO2. After the incubation phase 20µl of a 400µM Reaszurin solution was added to the cells and after 4h the viability was determined by fluorescence measurement with a Tecan Safire2. Based on the dose-dependent viability the IC50 of each reference substance was calculated using GraphPad Prism.

LIMITED USE

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

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