

## PRODUCT SPECIFICATION

### instaCELL Cytotoxicity Assay Kit

**CatN°:** SF020-01

**Lot#:** CX-230203

**Expiry Date:** 01.01.2024

#### 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	<b>1.07E+07</b>	9.00E+06<>1.20E+07
Homogeneity (cell count)	<b>98%</b>	≥ 90%
Viability (after thawing)	<b>96%</b>	≥ 90%
Proliferative Capacity	<b>98%</b>	≥ 70%
Debris/Cell Ratio	<b>0.3</b>	≤ 1.0
Aggregation	<b>1.2</b>	≤ 2.0
Sterility (bacteria, yeast, fungi)	<b>passed</b>	negative after 7 days
Sterility (mycoplasma)	<b>passed</b>	negative by PCR
Morphology	<b>passed</b>	unaltered to reference
Cytotoxicity Assay (max signal)	<b>26903 RFU</b>	15000 RFU < result > 25000 RFU
Cytotoxicity Assay (min signal)	<b>1073 RFU</b>	1000 RFU < result > 4000 RFU
Cytotoxicity Assay (IC50)	<b>Sodium Selenite: 2.8E-05M</b>	1.0E-05 M < x < 2.0E-04 M
Cytotoxicity Assay (Z')	<b>0.96</b>	> 0.5

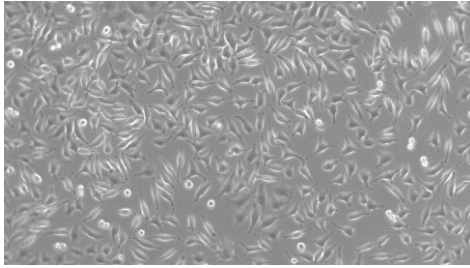
#### KIT CONTENT

	Lot#	Storage
Recovery Buffer A	<b>91-230104ML01</b>	<b>-20°C</b>
Assay Buffer A	<b>91-230202ML01</b>	<b>-20°C</b>
Assay Medium A	<b>91-230105ML01</b>	<b>-20°C</b>
Cytotoxic Control	<b>91-230106ML06</b>	<b>-20°C</b>
Resazurin Solution	<b>91-220727MD01</b>	<b>-20°C</b>
96-well Assay Plate	<b>I181673G</b>	<b>RT</b>
Assay Ready L-929 Cells	<b>92-220328NR01</b>	<b>&lt; -140°C</b>

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.

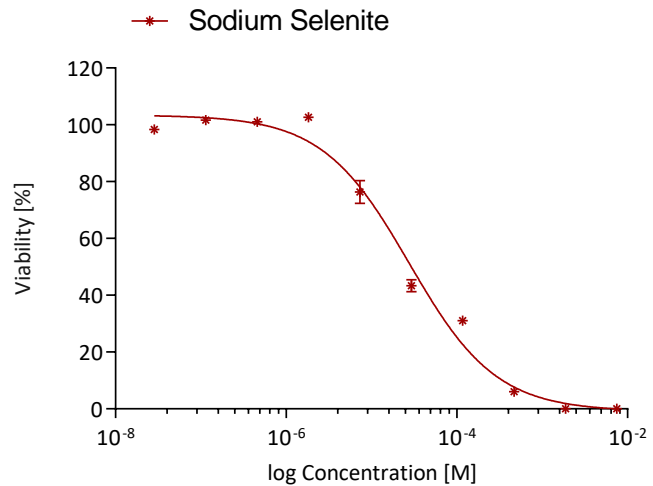


## MORPHOLOGY



Morphology of cells 24 hours after seeding

## CYTOTOXICITY ASSAY



Dose response of the reference compound Sodium Selenite (Cytotoxic Control) 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  $\mu$ l 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  $\mu$ l 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 a 96-well plate and treated with the reference substance Sodium Selenite for 24h at 37°C and 5% CO<sub>2</sub>. After the incubation phase 20 $\mu$ l of a 400 $\mu$ M Resazurin 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.

