

# CERTIFICATE OF ANALYSIS

## instaCELL Mikronucleus Assay Kit

**CatN°:** SF120-01

**Lot#:** MN-03052022

**Expiry Date:** 17.12.2022

### PRODUCT DEFINITION

Test kit to assess the genotoxic effect of chemicals and leachables by their application to cultures of V79 cells and the subsequent determination of number of nuclei and cell viability.

### QUALITY SPECIFICATION OF THE CELLS

	Batch Quality Control	Specification Limits
Cell Count	<b>5.83E+05</b>	4E+05<>6E+05
Homogeneity (cell count)	<b>97%</b>	≥ 90%
Viability (after thawing)	<b>98%</b>	≥ 90%
Proliferative Capacity	<b>90%</b>	≥ 70%
Debris/Cell Ratio	<b>0.1</b>	≤ 1.0
Aggregation	<b>1.6</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
Responsivity (0.6µM Mitomycin C)	<b>17 micronuclei</b>	≥ 10 micronuclei

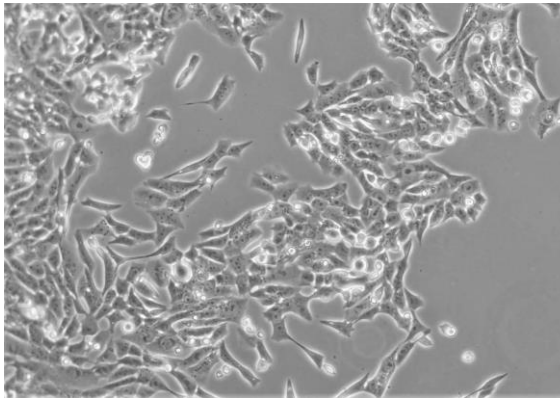
### KIT CONTENT

	Lot#	Storage
Recovery Buffer B	<b>91-210208NR02</b>	<b>-20°C</b>
Assay Buffer B	<b>91-211021NR02</b>	<b>-20°C</b>
Assay Medium B	<b>91-201217NR01</b>	<b>-20°C</b>
Serva, Mitomycin C	<b>200904</b>	<b>RT</b>
Flouroshield™	<b>91200923NR01</b>	<b>4°C</b>
Assay Ready V79 Cells	<b>92-200421VE01</b>	<b>&lt; -140°C</b>
3-well Ibidi chamber	<b>200901/4</b>	<b>RT</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 V79 Assay Ready Cells

## 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).
- Functional Testing:** Assay Ready Cells were thawed, washed once in 10 ml recovery buffer and seeded into a 3-well chamber slide at a density of  $6E+03$  c/cm<sup>2</sup>. Incubation for 24 h at 37 °C and 5 % CO<sub>2</sub>. The supernatant was discarded after adherence of the cells and replaced by positive control Mitomycin C (0.2 µg/ml). After 16 h of incubation at 37 °C and 5 % CO<sub>2</sub>, the cells were fixed and stained with Fluoroshield for microscopic analysis. To determine Genotoxicity and Viability a total of 2.000 cells was counted.

## 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. The cell line uses Luciferase technology from Promega (U.S. Pat No. 8008006 & EU Pat. No. 1341808B1). The Kit may only be used under the terms of a limited use license which is attached as part of this kit.

