

instaCELL micronucleus assay kit

Product Information

Cat N° SF120-01

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1 Description

The determination of genotoxicity represents an essential element for the safety assessment of chemicals. A battery of *in vitro* tests is available for this issue, including the micronucleus test using rodent cell lines like V79 [1]. DNA damage can result in double-strand breaks, the formation of chromosome fragments or even result in the loss of entire chromosomes. During mitosis, these fragments are enclosed separately and form micronuclei next to the daughter nucleus (Fig. 4). The instaCELL micronucleus assay kit provides a comprehensive basis for investigating chromosome damage potential because both aneugens and clastogens can be detected (Fig. 2).



Figure 1: instaCELL micronucleus assay kit

The instaCELL micronucleus assay kit includes prequalified Assay Ready V79 Cells as well as media, Fluoroshield™ with DAPI, controls, and 3-well chamber slides (Ibidi) to perform the assay according to the OECD 487 [2]. Assay Ready Cells are frozen aliquots of cells and can be used like a reagent, without prior cultivation directly in the assay.

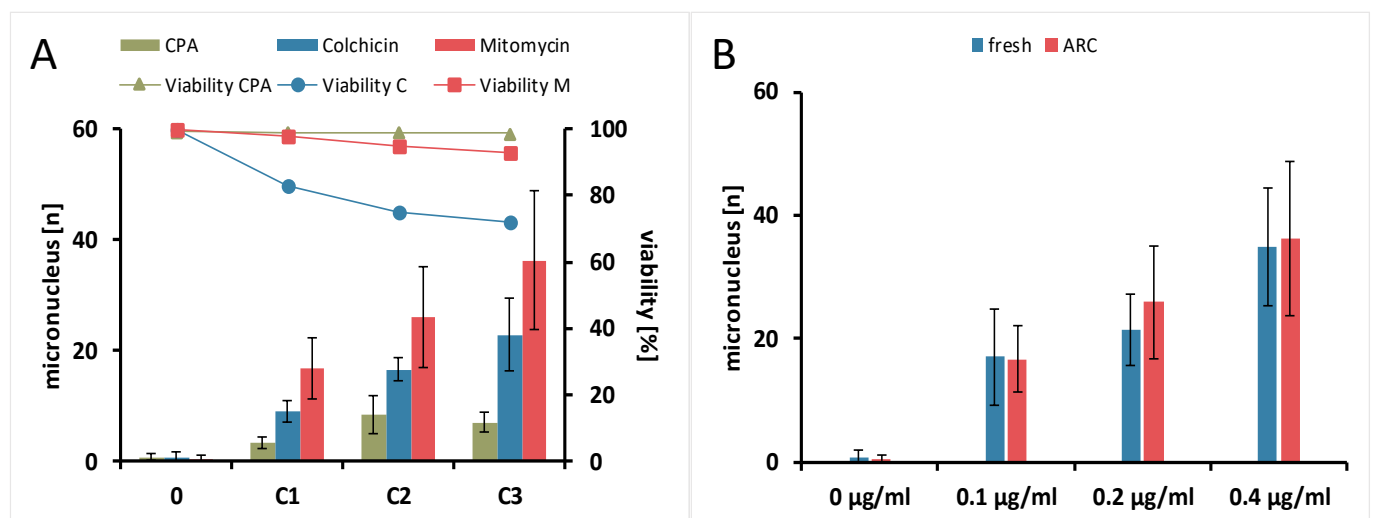


Fig. 2: Comparison of Assay Ready Cells with cells from a continuous culture and reaction to clastogens and aneugens

[A] The OECD guideline recommends assessing laboratory proficiency by using a set of chemicals, which cause chromosome breaks (clastogen) or interfere with spindle formation during mitosis (aneugen). Mitomycin C (clastogen), Colchicine (aneugen), and Cyclophosphamide (clastogen after activation) were used to verify the capability of the instaCELL micronucleus assay kit. A dose-dependent increase in the micronucleus rate was found for all three chemicals.

[B] Assay Ready Cells display no difference compared cells from a continuous culture when treated with a genotoxic substance like Mitomycin C. In both cases a clear dose-dependent increase in micronuclei formation can be observed.

2 Cell Information

Cell Type:	Fibroblast
Tissue:	Lung
Species:	Hamster
Morphology:	Fibroblast
Growth:	Adherent
Biosafety Level:	1

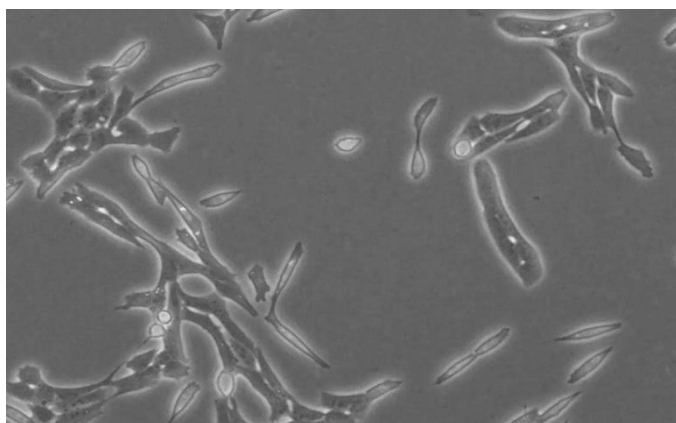


Figure 3: Morphology of KeratinoSens® Assay Ready Cells

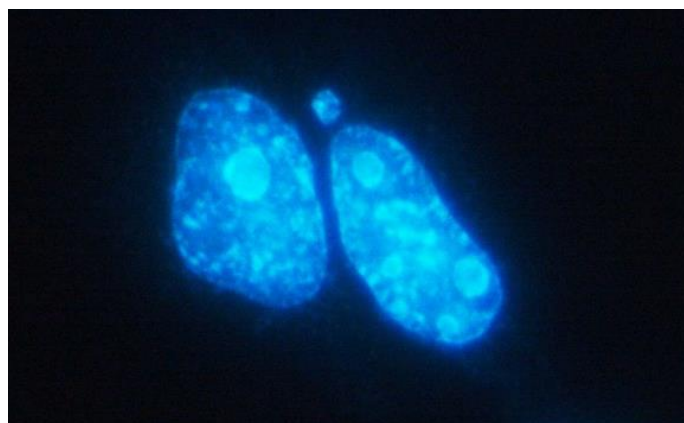


Figure 4: Micronucleus after cell division.

3 Kit Content

V79 Assay Ready Cells	1 vial (2.5E6 cells)	RE781K
Recovery Buffer B	1 bottle (10ml)	MD125-01
Assay Medium B	1 bottle (60ml)	MD425-06
Assay Buffer B	1 bottle (60ml)	MD325-06
Ibidi 3-well Chamber Slides	5 Slides	ZG16-01
Mitomycin C (Serva)	1 vial (500µg)	002980550
Fluoroshield	1 bottle (1ml)	RX702-01

Additionally required but not provided with the kit:

Methanol, Acetic Acid, 60x24mm coverslip

4 Protocol of Use

4.1 Day I: Seeding of V79 Cells

- Keep the cells on dry ice before thawing and process quickly.
- Equilibrate all media and buffer to 37°C.
- Thaw the Assay Ready Cells in a water bath at 37°C for 3min. Prepare 9ml of recovery buffer in a 15ml centrifuge tube. Dispense the cells completely into the prepared tube. Rinse the cryovial once with 1ml recovery buffer.
- Centrifuge for 3min at 200xg and carefully aspirate the supernatant, flick the centrifuge tube to loosen the cell pellet. Resuspend the cells in 5ml of assay medium.
- Dilute 2ml of cell suspension into 18ml of assay medium to obtain the working cell density.
- Dispense 1ml of the working cell suspension into each well of the five provided microscopy slides.
- Incubate for 24h in a humidified incubator at 37°C and 5% CO₂.

4.2 Day II: Preparation and addition of test sample and controls to the cells

- Prepare six serial dilutions of the test chemical in assay buffer.
- Dissolve Mitomycin C in 1ml assay buffer (1.5mM). Prepare the positive control (0.6µM) by diluting the Mitomycin solution twice 1:50.
- Aspirate the assay medium. Add 1ml of test chemical dilutions (as duplicates) and controls to the cells.
- Incubate for 16h in a humidified incubator at 37°C and 5% CO₂.

4.3 Day III: Readout by Resazurin Viability Assay

Cool down 20ml methanol to -20°C.

- Prepare fixation solution, by adding 2.5ml acetic acid to 7.5ml methanol.
- Aspirate test chemicals and fix the cells by rinsing each well once with 500µl fixation solution.
- Wash each well first with 500µl ice cold methanol and second with 500µl ddH₂O.
- Remove the silicon sealing from each microscopy slide, add 4 drops of Fluoroshield™ with DAPI along the slide and mount the cells with a 60x24mm coverslip (avoid air bubbles).
- Leave the slides to dry for 10-15min at room temperature in the dark.

5 Analysis and Assay Acceptance Criteria

- Analyze 2.000 cells per well by fluorescence microscopy (340nmEx/488nmEm) for a valid test.
- Count the micronuclei and the apoptotic cells to determine the micronucleus rate and cell viability.

6 Stability & Storage

Performance of the kit is guaranteed within the specifications as defined in the certificate of analysis only before the expiration date as indicated on the packaging and only if stored and handled according to the instruction of this datasheet.

- Store Assay Ready Cells below -140°C (e.g., in vapor phase of liquid nitrogen).
- Store all other reagents and media as indicated on the label.

7 Literature and Related Documents

[1] von der Hude W, Kalweit S, Engelhardt G, McKiernan S, Kasper P, Slacik-Erben R, Miltenburger HG, Honarvar N, Fahrig R, Görlitz B, Albertini S, Kirchner S, Utesch D, Pötter-Locher F, Stopper H, Madle S. In vitro micronucleus assay with Chinese hamster V79 cells - results of a collaborative study with in situ exposure to 26 chemical substances. *Mutat Res.* 2000 Jul 10;468(2):137-63. doi: 10.1016/s1383-5718(00)00045-0. PMID: 10882892.

[2] OECD 487: In Vitro Mammalian Cell Micronucleus Test

8 Support

<https://www.accelerate.me/support/contact.html>

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9 Disclaimer

The product is sold under the terms of a Limited Use Label License attached to the product. By breaking the seal of the product package, the user explicitly agrees to the license terms. Assay Ready Cells are for immediate assay use only. The user shall not propagate, passage, or refreeze the cells.

This product is intended for research use only. Do not use for diagnostic or therapeutic purposes.