

# instaCELL® micronucleus assay kit protocol



Version 1.0  
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## kit content

- Assay Ready V 79 (1x vial)
- Assay Buffer B (1x bottle)
- Assay Medium B (1x bottle)
- Recovery Buffer B (1x bottle)
- Mitomycin C (1x vial)
- Fluoroshield™ (1x bottle)
- Ibbidi® 3 well chamber (5x)

## not provided

- Methanol
- Acetic Acid
- 60x24mm coverslip

## storage

- Store Assay Ready Cells in liquid nitrogen (below -140°C)
- Store all reagents and media at -20°C
- Store Fluoroshield™ at 4°C

## limited product warranty

This warranty limits our liability to replace this product. acCELLerate does not provide any other warranties of any kind, expressed or implied. Without limitation, this includes implied warranties of merchantability or fitness for a particular purpose. acCELLerate shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product. The product is for research only and must not be used for diagnostic or therapeutic purposes.

## limited use license

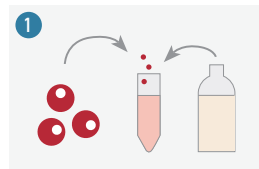
Assay Ready Cells are provided under a limited use license and shall only be used instantly for the particular purpose of the instaCELL bioassay kit. No further expansion, passaging or refreezing of the cells is permitted. When breaking the sealed bag of Assay Ready Cells, the user is explicitly accepting the terms of this limited use license.

Fluoroshield™ is a Trademark of ImmunoBioScience corp.  
instaCELL® is a registered trademark of acCELLerate GmbH.

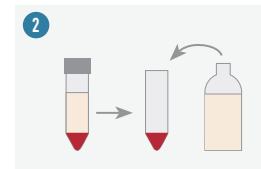


SAFETY

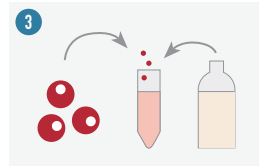
please@accelerate.me  
www.accelerate.me



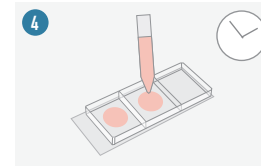
1 thaw cells for 2min at 37°C, dilute in 9ml recovery buffer



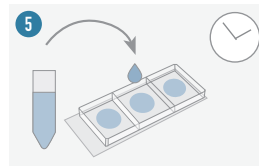
2 centrifuge for 3min at 100xg, resuspend in 5ml assay medium



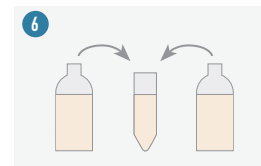
3 dilute cells 1:10



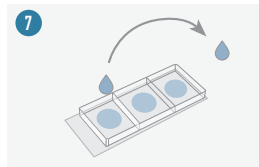
4 add 1ml cell solution to each well, incubate 24h



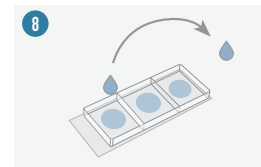
5 aspirate medium and add 1ml of test chemical dilutions and controls, incubate 16h



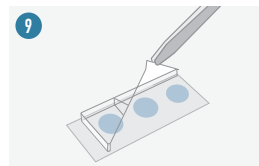
6 prepare acetic acid/methanol (1:4)



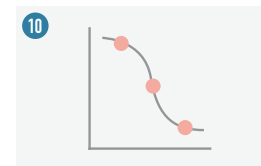
7 aspirate test chemical and rinse with 500µl fixation solution



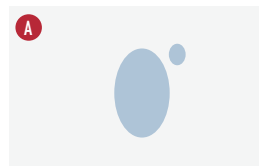
8 wash first with 500µl methanol and second with 500µl ddH<sub>2</sub>O



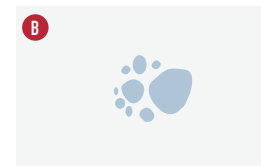
9 remove sealing and mount with Fluoroshield™



10 analyse 2.000 cells per well



A nucleus with a micronucleus



B fragmented nucleus of an apoptotic cell

## day I: preparation of cells

- Keep the cells on dry ice before thawing and process quickly.
- Equilibrate all media and buffer to room temperature.
- Thaw one vial of Assay Ready Cells in a water bath at 37°C for 2min. Prepare 9ml of recovery buffer in a 15ml centrifugation tube and transfer the cells completely into the tube. ①
- Centrifuge for 3min at 100xg and carefully aspirate the supernatant. Resuspend the cell pellet in 5ml of assay medium. ②
- Dilute 2ml of cell suspension into 18ml of assay medium to obtain the working cell density. ③
- 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% CO<sub>2</sub>. ④

## day II: preparation of test chemicals and incubation

- 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% CO<sub>2</sub>. ⑤

## day IV: staining and microscopic analysis

- Cool down 20ml methanol to -20°C.
- Prepare fixation solution, by adding 2.5ml acetic acid to 7.5ml methanol. ⑥
- Aspirate test chemicals and fix the cells by rinsing each well once with 500µl fixation solution. ⑦
- Wash each well first with 500µl ice cold methanol and second with 500µl ddH<sub>2</sub>O. ⑧
- 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.
- Analyse 2.000 cells per well by fluorescence microscopy (340nm<sub>ex</sub>/488nm<sub>em</sub>) for a valid test. ⑩
- Cells with a micronucleus **A** such as apoptotic cells **B**, are counted separately in order to determine the micronucleus rate and viability.

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