acCELLerate®

instaCELL® micronucleus assay kit

protocol

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)
- Ibidi® 3 well chamber (5x)

not provided

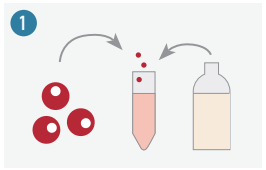
- Methanol
- Acetic Acid
- 60x24mm coverslip

storage

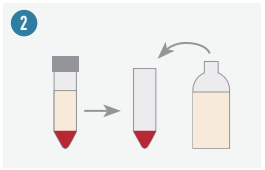
- Store Assay Ready Cells in liquid nitrogen (below -140°C)
- Store all reagents and media at -20°C
- Store Fluoroshield™ at 4°C

SAFETY

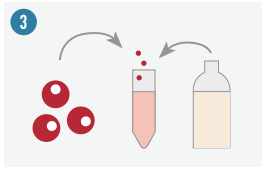
please@accellerate.me
www.accellerate.me



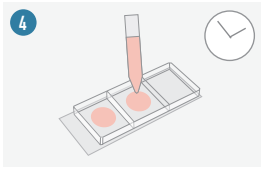
thaw cells for 2min at 37°C, dilute in 9ml recovery buffer



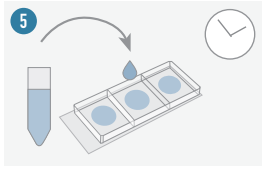
centrifuge for 3min at 100xg, resuspend in 5ml assay medium



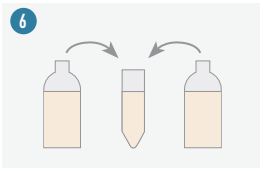
dilute cells 1:10



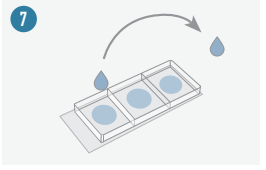
add 1ml cell solution to each well, incubate 24h



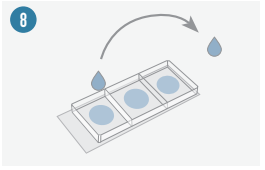
aspirate medium and add 1ml of test chemical dilutions and controls, incubate 16h



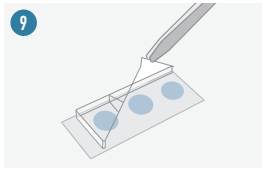
prepare acetic acid/methanol (1:4)



aspirate test chemical and rinse with 500µl fixation solution



wash first with 500µl methanol and second with 500µl ddH_2O



remove sealing and mount with Fluoroshield™

analyse 2.000 cells per well

nucelus with a micronucleus

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_2. 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_2. 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_2O. 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$_{Ex}$/488nm$_{Em}$) 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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