instaCELL® KeratinoSens® assay kit

protocol

Version 1.0

kit content

- Assay Ready Cells (1x vial)
- Assay Buffer H (1x bottle)
- Assay Medium H (1x bottle)
- Recovery Buffer H (1x bottle)
- Resazurin (1x bottle)
- Promega, One-Glo™ (1x)
- pos. control EGDMA (1x vial)
- 96 well plates (2x)

not provided

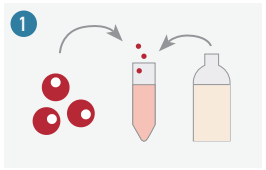
- 50ml centrifugation tube
- 96 well master plates
- DMSO
- DPBS

storage

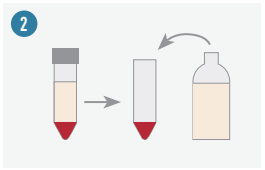
- Store Assay Ready Cells in liquid nitrogen (below -140°C)
- Store all reagents and media at -20°C

SAFETY

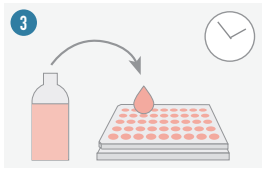
please@accellerate.me
www.accellerate.me



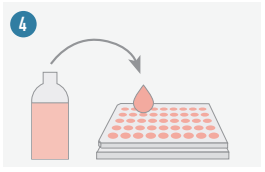
thaw cells for 2min at 37°C,
dilute in 9ml recovery buffer



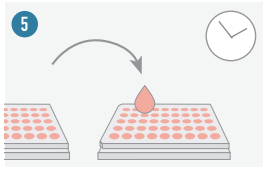
centrifuge for 3min at 100xg,
resuspend in 30ml assay medium



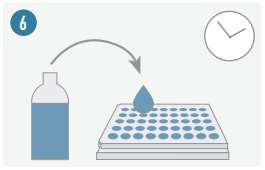
dispense cells 125 µl/well,
incubate for 24h



aspirate medium,
add 150 µl/well assay buffer

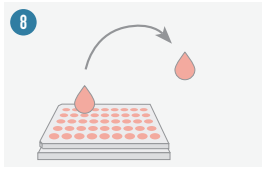


transfer 50µl of diluted chemicals
to each corresponding well,
incubate for 48h

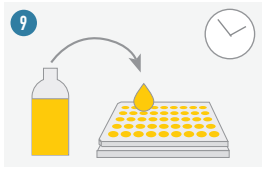


add Resazurin 20 µl/well,
incubate for 4h

measure Fluorescence
at 540_{Ex}/ 590_{Em}



aspirate supernatant, wash each
well once with 100µl DPBS



add 50µl DPBS and 50µl One-Glo™
to each well, incubate for 20min

measure luminescence with
1s integration time

The Manual is based on the OECD testguideline 442D,
For more details please refer to the DB-ALM protocol n° 155.

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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 50ml centrifugation tube. Dispense the cells completely into the prepared tube. 1
- Centrifuge for 3min at 100xg and carefully aspirate the supernatant. Resuspend the cell pellet in 30ml of assay medium. 2
- Dispense 125µl of the cell suspension into each well of the provided assay plates except the wells reserved for blank values. Refer to the recommended plate layout and evaluation sheet on www.accellerate.me/support/downloads. 3

day II: preparation of test chemicals and incubation with cells

- Dissolve the test chemicals in DMSO to a stock concentration of 200mM.
- Prepare serial dilutions from the stock solutions in DMSO, to obtain twelve master concentrations of the test chemicals and five of the provided positive control. Dilute the master concentrations 25-fold into assay buffer.
- Aspirate medium from the assay plates and dispense 150µl of assay buffer into each well. 4
- Add 50µl of the diluted test chemicals and controls to the corresponding wells of the assay plates. Use assay buffer, containing 1% DMSO, as solvent control.
- Incubate for 48h in a humidified incubator at 37°C and 5% CO_2. 5

day IV: staining and read-out

- Add 20µl of resazurin to each well and incubate for 4h at 37°C. 6
- Measure the fluorescence on a plate reader at $540_{Ex}/590_{Em}$ to determine the viability of the cells. 7
- Equilibrate all One-Glo™ components to room temperature. Reconstitute the One-Glo™ Reagent by adding 10ml of the Luciferase Assay Buffer to the Luciferase Assay Substrate.
- Aspirate the supernatant of each well. Wash the cells once with 100µl DPBS. 8
- Dispense 50µl of DPBS and 50µl of One-Glo™ Reagent to each well and incubate for 20min at room temperature in the dark. 9
- Measure luminescence with an integration time of 1 s/well. 10

assay acceptance criteria

- Dose-dependent increase in luciferase induction, at least 2-fold above the solvent control.
- An $EC_{1.5}$ between 30-100µM.