kit content

- instaCELLs (2x vial)
- Assay Buffer H
- Assay Medium H
- Recovery Buffer H
- Resazurin
- pos. Control EGDMA
- Promega, One-Glo™
- 96-well assay plate
- sealing tape

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

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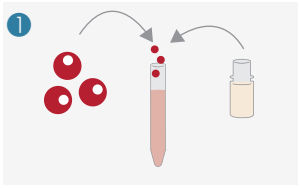
acCELLerate®

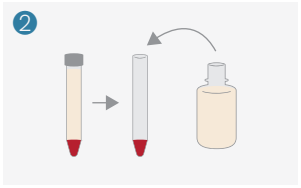
instaCELL® KeratinoSens® assay kit
multiplex protocol

SAFETY

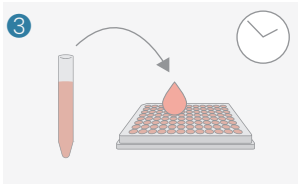
Version 3.0



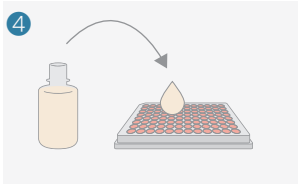
thaw cells for 2min at 37°C, dilute in 9ml recovery buffer



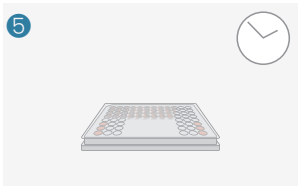
centrifuge for 3min at 200xg, resuspend in 15ml assay medium



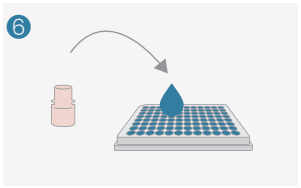
dispense cells 125µl/well, incubate for 24h



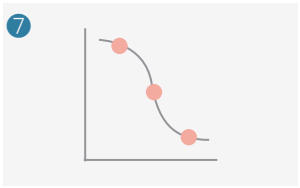
aspirate medium, add 150µl/well assay buffer



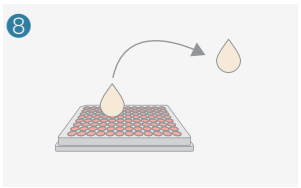
transfer 50µl of diluted chemicals to each corresponding well, incubate for 48h



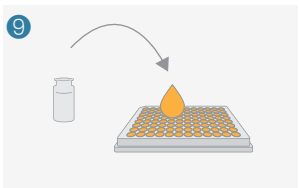
add Resazurin 20µl/well, incubate for 4h



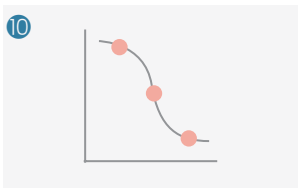
measure fluorescence at $540_{Ex}/590_{Em}$



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

day I: preparation of cells

- Keep the cells on dry ice before thawing and process quickly.
- Equilibrate all media and buffers to room temperature.
- Thaw one vial of instaCELLs 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 200xg and carefully aspirate the supernatant. Resuspend the cell pellet in 15ml of assay medium. 2
- Dispense 125µl of the cell suspension into each well of one provided assay plate, except the wells reserved for blank values.
- Incubate the cells for 24h in a humidified incubator at 37°C and 5% CO_2. 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 plate 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 plate. Use assay buffer, containing 1% DMSO, as solvent control.
- Cover the plate with sealing tape and incubate for 48h in a humidified incubator at 37°C and 5% CO_2.

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 obtained with positive control, at least 2-fold above the solvent control for highest control concentration.
- An $EC_{1.5}$ between 30-100µM.