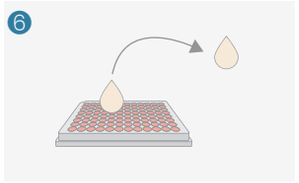
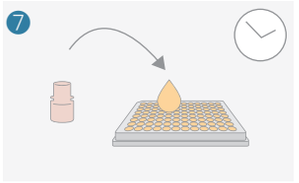
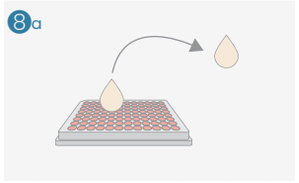
viability



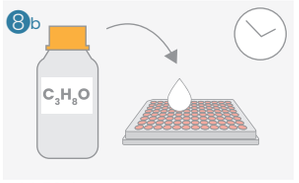
aspirate supernatant from the clear well 96-well plate



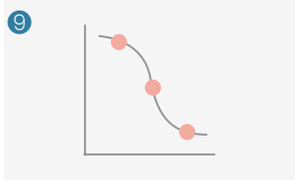
add 200µl MTT solution to each well, cover the plate with sealing tapes, incubate for 4h at 37°C



aspirate supernatant from the clear well 96-well plate

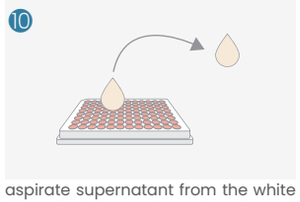


add 50µl isopropanol and incubate for 30min at RT in the dark

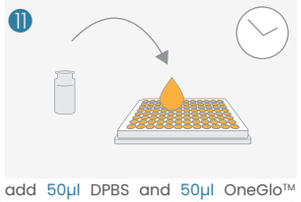


measure the absorption at 570nm

luminescence



aspirate supernatant from the white 96-well plate, wash each well once with 150µl DPBS



add 50µl DPBS and 50µl OneGlo™ to each well, incubate for 20min at room temperature in the dark

measure luminescence with 1s integration time

day IV: staining and read-out (viability)

- Carefully remove the supernatant of the clear plate. 6
- 2.7ml MTT is added to 20ml assay buffer H.
- Add 200µl of the MTT solution to each well, cover the plate with sealing tape and incubate for 4h at 37°C. 7
- After the incubation, the medium is removed and 50µl isopropanol is added. Incubate the plate at room temperature for 30min in the dark. 8
- Measure the absorption on a plate reader at 570nm to determine the viability of the cells. 9

day IV: staining and read-out (luminescence)

- 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 of the white 96-well plate. Wash the cells once with 125µl DPBS. 10
- Dispense 50µl of DPBS and 50µl of One-Glo™ reagent to each well and incubate for 20min at room temperature in the dark. 11
- Measure luminescence with an integration time of 1s/well. 12

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.

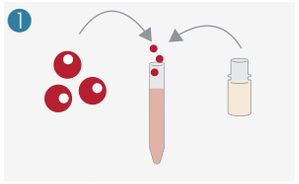
acCELLerate

CatN° SF220-02

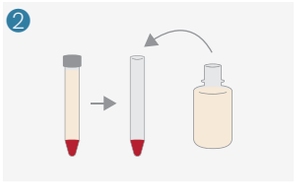
instaCELL KeratinoSens assay kit
protocol

SAFETY

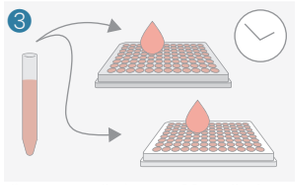
Version 1.0



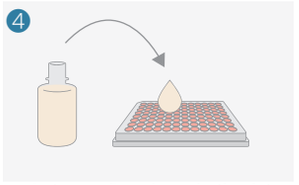
thaw cells for 3min at 37°C, dilute in 8ml recovery buffer



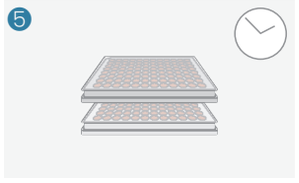
centrifuge for 3min at 200xg, resuspend in 30ml assay buffer



dispense cells in two 96-well plates with 125µl/well, incubate for 24h



aspirate medium from both plates, add 150µl/well assay buffer



transfer 50µl of diluted chemicals to each corresponding well, cover the plate with sealing tapes, incubate for 48h

day I: preparation of cells

- Keep the cells on dry ice before thawing and process quickly.
- Equilibrate all media and buffer to 37°C.
- Thaw two vials of instaCELLs in a water bath at 37°C for 3min.
- Prepare 8ml 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 30ml of assay medium. 2
- Dispense 125µl of the cell suspension into each well of one provided assay plates, except the wells reserved for blank values. One clear 96-well plate and one white plate.
- Incubate for 24h in a humidified incubator at 37°C and 5%CO_2. 3

Refer to the recommended plate layout and evaluation sheet on www.accellerate.me/support/downloads.

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 both 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 plate. Use assay buffer, containing 1% DMSO, as solvent control.
- Cover the plates with sealing tape and incubate for 48h in a humidified incubator at 37°C and 5%CO_2. 5

kit content

- instaCELLs (4x vials)
- 2x Positive control
- Promega One-Glo™
- 2x Assay buffer H
- 2x Transparent 96-well plates
- 2x Assay medium H
- 2x White 96-well plates
- 2x Recovery buffer H
- 1x MTT solution
- 4x Sealing tape

not provided

DMSO
DPBS
50ml centrifugation tube
96-well master plates

storage

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

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