## viability

well 96-well plate

9



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

8



aspirate supernatant from the clear well 96-well plate





# day IV: staining and read-out (viability)

- Carefully remove the supernatant of the clear plate.
- 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.
- 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.

## 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. 🚺
- Dispense 50µl of DPBS and 50µl of One-Glo™ reagent to each well and incubate for 20min at room temperature in the dark.
- Measure luminescence with an integration time of 1s/well. 😰

# 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 EC1.5 between 30–100µM.



# CatN° SF220-02

# instaCELL KeratinoSens assay kit protocol





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



integration time

measure luminescence with 1s



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





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



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



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



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



aspirate medium from both plates, add 150ul/well assav buffer

#### 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.
- · 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<sub>2</sub>.

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.
- 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<sub>2</sub>. (5)

### kit content



#### not provided

DMSO	50ml centrifugation tube
DPBS	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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