

instaCELL® KeratinoSens® assay kit protocol



Version 1.0
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kit content

- Assay Ready Cells (2x vials)
- 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

limited product warranty

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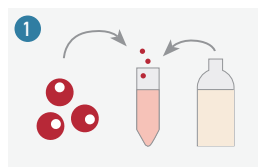
limited use license

The cell line uses the Luciferase technology from Promega (U.S. Pat. No. 8008006 & EU Pat. No. 1341808B1). The cells may only be used under the terms of a limited use license which is attached as part of this kit.

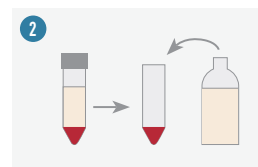
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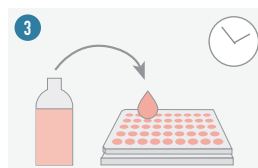
please@accelerate.me
www.accelerate.me



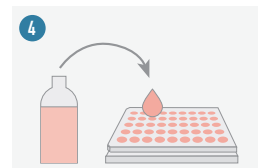
thaw cells for **2min** at **37°C**,
dilute in **9ml** recovery buffer



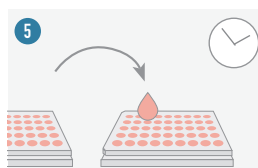
centrifuge for **3min** at **100xg**,
resuspend in **15ml** assay medium



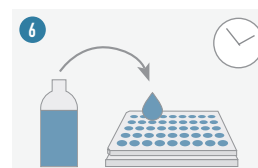
dispense cells **125 µl/well**,
incubate for **24h**



aspirate medium,
add **150 µl/well** assay buffer



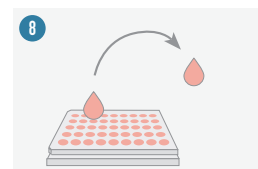
transfer **50µl** of diluted chemicals
to each corresponding well,
incubate for **48h**



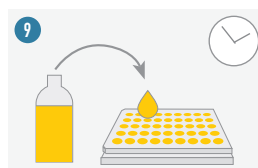
add Resazurin **20 µl/well**,
incubate for **4h**



measure Fluorescence
at **540Ex/ 590Em**



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 **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. Refer to the recommended plate layout and evaluation sheet on www.accelerate.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% CO₂**. **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 **540Ex/590Em** 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**.