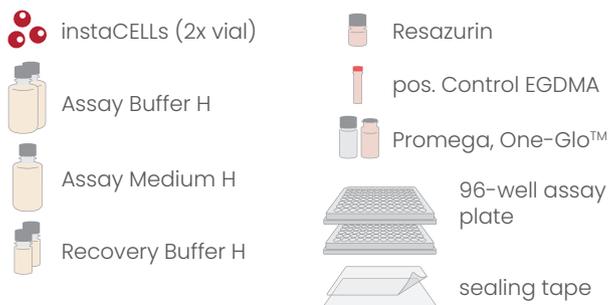


kit content



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

This warranty limits our liability to replace this product. acCELLerate does not provide any other warranties of any kind, expressed or implied. Without limitation, this includes implied warranties of merchantability or fitness for a particular purpose. acCELLerate shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product. The product is for research only and must not be used for diagnostic or therapeutic purposes.

limited use license

Assay Ready Cells are provided under a limited use license and shall only be used instantly for the particular purpose of the instaCELL bioassay kit. No further expansion, passaging or refreezing of the cells is permitted. When breaking the sealed bag of Assay Ready Cells, the user is explicitly accepting the terms of this limited use license.

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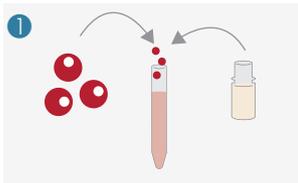
please@accellerate.me
www.accellerate.me

instaCELL® KeratinoSens® assay kit multiplex protocol

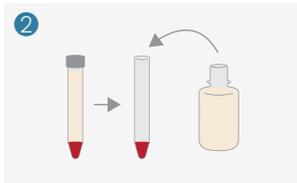


SAFETY

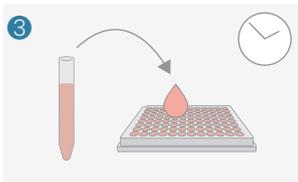
Version 3.0
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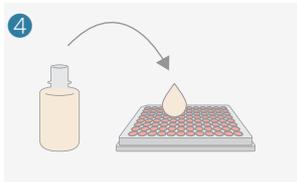
thaw cells for **2min** at **37°C**, dilute in **9ml** recovery buffer



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



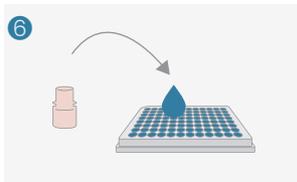
dispense cells **125µl/well**, incubate for **24h**



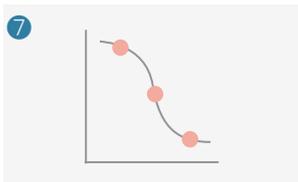
aspirate medium, add **150µl/well** assay buffer



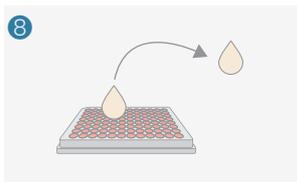
transfer **50µl** of diluted chemicals to each corresponding well, incubate for **48h**



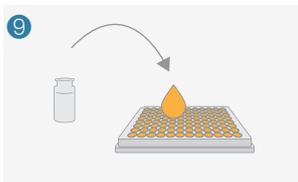
add Resazurin **20µl/well**, incubate for **4h**



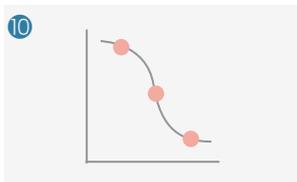
measure fluorescence at **540_{Ex}/590_{Em}**



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% CO₂**. **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% CO₂**.

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 **1s/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 **EC₁₅** between **30-100µM**.