

## kit content



## not provided

DMSO

## storage

Store Assay Ready Cells in liquid nitrogen (below  $-140^{\circ}\text{C}$ )  
Store all reagents and media at temperatures indicated on the label

+

## limited product warranty

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## 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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## instaCELL® G-CSF potency assay kit protocol



thaw

dispense

add samples

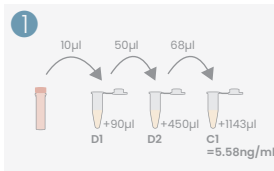
incubate

measure

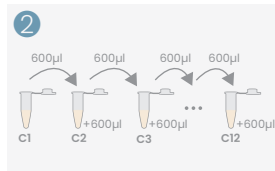


QUALITY

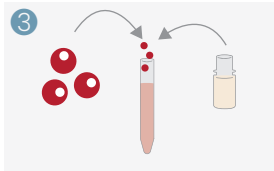
Version 1.0  
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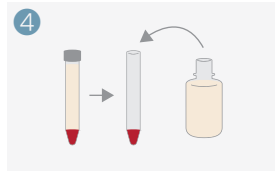
dilute sample and controls



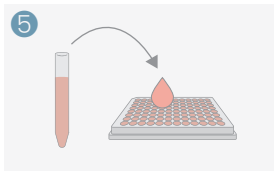
prepare sample and control concentrations



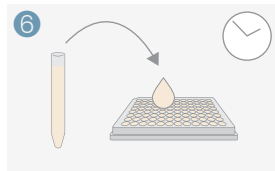
thaw cells for 3min at 37°C, dilute in 8ml recovery buffer



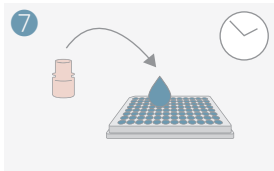
centrifuge for 3min at 200xg, resuspend in 11ml assay buffer



dispense cells 75 µl/well



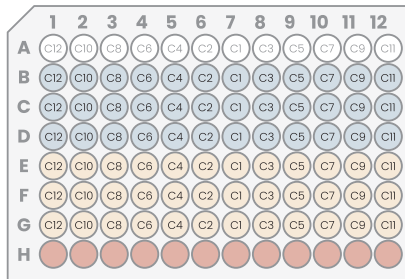
add 75µl of reference, sample and control to each corresponding well, incubate for 48h



add Resazurin 20 µl/well, incubate for 4h



measure Fluorescence at 540<sub>ex</sub>/590<sub>em</sub>



- blank control, no cells
- reference concentration
- sample concentration
- medium control

### day I.a: preparation of test sample and controls

To avoid edge effects, the space between the wells of the assay plate can be filled with sterile liquid (e.g. PBS).

- Equilibrate assay buffer to room temperature.
- Dilute the G-CSF reference **two times 10-fold** in assay buffer. First 10µl stock solution in 90µl assay buffer (D1) and then 50µl of D1 in 450µl assay buffer (D2).
- Further dilute 68µl of D2 in 1143µl assay buffer to obtain the top concentration (C1) of the G-CSF reference standard. This solution is 2x concentrated and contains 5.58 ng/ml. **1**
- Perform a 2-fold dilution series of 12 concentrations for the G-CSF reference standard curve. Add 600µl of C1 to 600µl of assay buffer, mix well and continue this process up to C12.
- Prepare a 2x concentrated dilution series of your test sample in assay buffer, analogue to the G-CSF standard dilution series. **2**

### day I.b: preparation of cells

- Keep the cells on dry ice before thawing and process quickly.
- Equilibrate all media and buffer to 37°C.
- Thaw one vial of Assay Ready Cells in a water bath at 37°C for 3min. Prepare 8ml of recovery buffer in a 15ml centrifuge tube. Dispense the cells completely into the prepared tube. Rinse the cryovial once with 1ml recovery buffer. **3**
- Centrifuge for 3min at 200xg and carefully aspirate the supernatant. Resuspend the cell pellet in 11ml of assay buffer. **4**
- Dispense 75µl of the cell suspension into each well of the provided assay plate, except row A. Mix cell suspension before each pipetting step. **5**
- Add 75µl of G-CSF reference (rows A-D) and sample (rows E-G) dilutions in triplicate to the respective wells as recommended in the plate layout. The plate layout has an inside-out design to avoid edge effects in relevant concentrations.
- Add 75µl of assay buffer to each well of row A and row H for blank and medium control. **6**
- Incubate for 48h in a humidified incubator at 37°C and 5% CO<sub>2</sub>.

### day III: Resazurin viability assay

- Equilibrate resazurin solution to room temperature.
- Add 20µl of resazurin solution to each well of the assay plate and shake for 15s on an orbital shaker. **7**
- Incubate for 4h in a humidified incubator at 37°C and 5% CO<sub>2</sub>.
- Shake the plate again for 15s on an orbital shaker. Afterwards measure the fluorescence at 540<sub>ex</sub>/590<sub>em</sub>. **8**