kit content

- NFS-60 Assay Ready Cells (1x vial)
- Resazurin Solution
- Assay Buffer G
- G-CSF Reference
- Recovery Buffer G
- 96-well plate

not provided

DMSO

storage

Store Assay Ready Cells in liquid nitrogen (below -140°C)
Store all reagents and media at temperatures indicated on the label

+

EUROPEAN OFFICE
+49 (160) 987 577 56
acCELLerate GmbH
Osterfeldstraße 12-14
22529 Hamburg
Germany

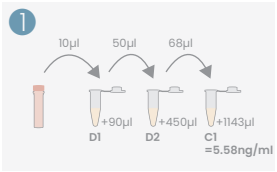
US OFFICE
+1 (732) 698 34 04
acCELLerate, Inc.
400 Route 518
Skillman, NJ 08558
USA

please@accellerate.me
www.accellerate.me

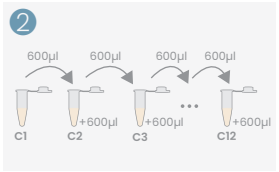
acCELLerate®

instaCELL® G-CSF potency assay kit
protocol

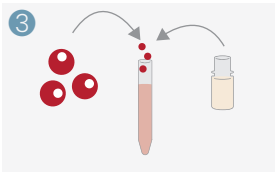
QUALITY



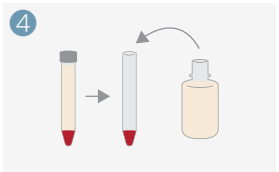
dilute sample and controls



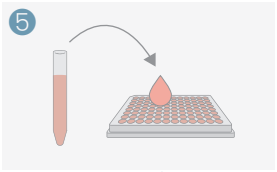
prepare sample and control concentrations



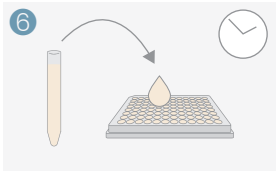
thaw cells for 3min at 37°C, dilute in 8ml recovery buffer



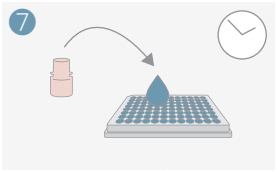
centrifuge for 3min at 200xg, resuspend in 11ml assay buffer



dispense cells 75 µl/well

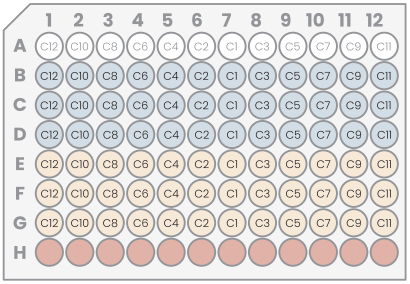


add 75µl of reference, sample and control to each corresponding well, incubate for 48h



add Resazurin 20 µl/well, incubate for 4h

measure Fluorescence at $540_{Ex}/590_{Em}$



- 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_2.

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_2.
- Shake the plate again for 15s on an orbital shaker. Afterwards measure the fluorescence at $540_{Ex}/590_{Em}$. 8