kit content

- CTLL-2 instaCELLs (1x vial)
- Assay Buffer L
- Recovery Buffer L
- Resazurin Solution
- IL-2 Reference Standard
- 96-well plate

not provided

15ml centrifuge tube, PBS

storage

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

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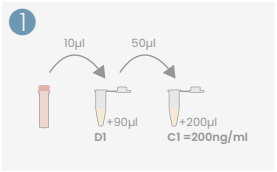
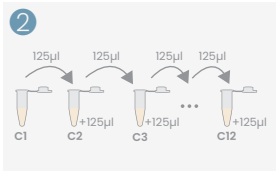
CatN° SF350-01

instaCELL IL-2 potency assay kit
protocol

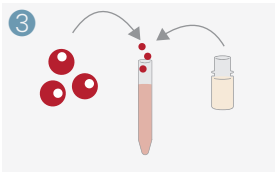
QUALITY



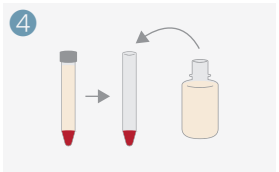
dilute the IL-2 stock solution



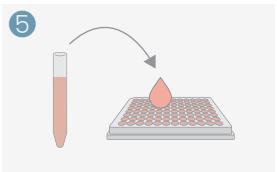
prepare sample an control concentrations



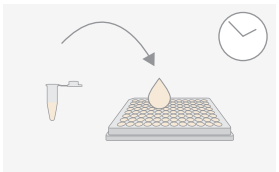
thaw cells for 2min at 37°C, dilute in 8ml recovery buffer



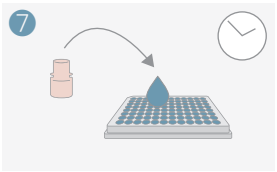
centrifuge for 5min at 400xg, resuspend in 11ml assay buffer



dispense cells 90µl/well

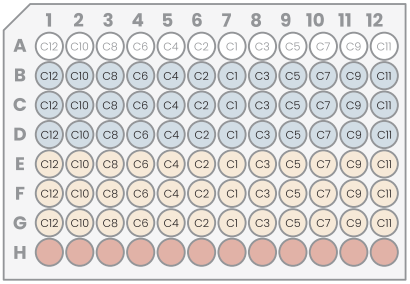


add 10µl of reference, sample and control to each corresponding well and incubate for 48h



add Resazurin 20 µl/well, incubate for 4h

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



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 IL-2 reference 10-fold in assay buffer. Add 10µl stock solution to 90µl assay buffer (D1).
- For the IL-2 dilution series, add 200µl Assay Buffer L to the first microtube and add 50µl of D1 to obtain C1 of the dilution series. 1
- Perform a 2-fold dilution series of 12 concentrations for the IL-2 reference standard curve. Add 125µl of C1 to 125µl 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 IL-2 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 2min. 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 5min at 400xg and carefully aspirate the supernatant. Resuspend the cell pellet in 11ml of assay buffer. 4
- Dispense 90µl of the cell suspension into each well of the provided assay plate, except row A. Mix cell suspension before each pipetting step. 5
- Add 90µl assay buffer to each well in row A.
- Add 10µl of IL-2 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. 6
- Shake the plate for 30sec on an orbital shaker.
- 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