Product Information

instaCELL IL-2 potency assay kit

Cat N° SF350-01

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Content

1 Description

Interleukin-2 (IL-2) has a central role in the regulation and activation of immune cells, particularly in the T-cell-mediated immune response. Because of its central function, IL-2 is used in several areas of medicine and research. Therapeutic applications, such as immunotherapy, require precise dosing of IL-2 to achieve optimal stimulation of the immune system. Therefore, a reliable assay for the determination of IL-2 activity is essential to ensure the efficacy and safety of biopharmaceutical products that contain or induce IL-2 production.

Figure 1: instaCELL IL-2 potency assay kit

The World Health Organization (WHO) has conducted and published a report of "Report on a Collaborative study for proposed 2nd International standard for Interleukin -2 (IL-2)". IL-2 can be potentiated, based on the International Standard, which has a fixed known effective size of units[1]. The CTLL-2 IL-2 Potency assay is based on the WHO publication and has been compared with the International Standard provided by the NIBSC.

The instaCELL IL-2 potency assay is an *in vitro* assay specifically designed for quality control to accurately determine the potency of IL-2. The assay uses CTLL-2 instaCELLs, a murine T cell line that is specific and sensitive to IL-2. These cells proliferate exclusively in the presence of IL-2, making them an ideal model for the determination of IL-2 activity. The instaCELL IL-2 potency assay provides an accurate and reliable method for the assessment of Cell viability, which is a direct measure of the proliferation of CTLL-2 instaCELLs, is measured using the resazurin assay. Resazurin is a non-toxic, cell-permeable dye that is reduced to Resorufin by the metabolic activity of living cells. Resorufin is fluorescent and the amount of this product directly correlates with the metabolic activity of the cells and thus with the concentration of IL-2 in the sample. Quantification is performed using an IL-2 reference standard to ensure accurate and reproducible results.

The instaCELL IL-2 potency assay kit includes prequalified Assay Ready CTLL-2 instaCELLs as well as media, reagents, IL-2 reference standard and 96-well plate to perform the assay. Assay Ready instaCELLs are frozen cell aliquots that can be used directly in the assay without prior cultivation, like a reagent.

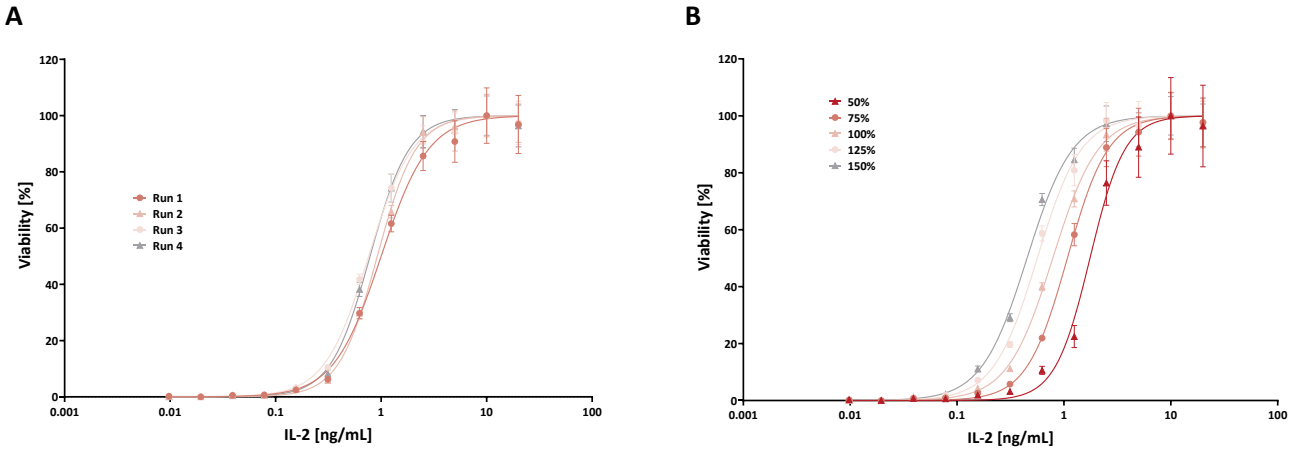

Figure 2: Precision & Accuracy of the instaCELL IL-2 potency assay kit

[A] The potency of a given IL-2 preparation can be precisely determined with CTLL-2 instaCELLs. In four independent experiments performed on different days using individual aliquots of CTLL-2 instaCELLs and it shows a coefficient of variation (CV) of 11% shows a very high intra-assay repeatability. **[B]** The accuracy of the IL-2 potency assay was tested using nominal doses of 50%, 70%, 100% and 150% measuring potencies of 44%, 72%, 100% and 139%.

2 Cell Information

Cell Type:	T-cell
Tissue:	Lymphoblast
Species:	Mouse
Growth:	suspension
Biosafety Level:	1

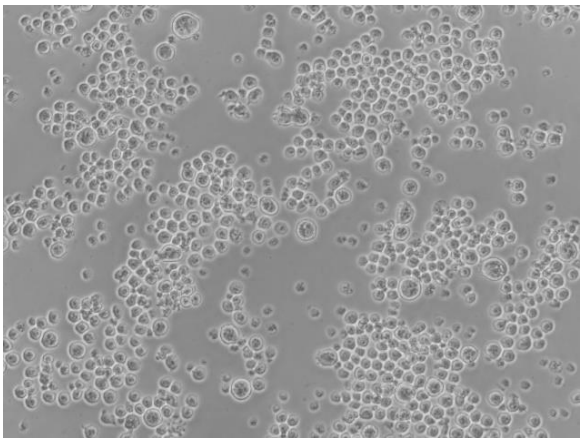

Figure 2: Morphology of CTLL-2 instaCELLs

3 Kit Content

• CTLL-2 instaCELLs	1 vial (6E+06 cells)	RE787K
• Recovery Buffer L	1 bottle (10ml)	MD166-01
• Assay Buffer L	1 bottle (60ml)	MD366-06
• IL-2 reference standard	1 vial (20µl)	RX521-01
• Resazurin	1 bottle (5ml)	RX718-01
• 96-well plate	1 plate	ZG02-13

Additionally required but not provided with the kit:

15 ml centrifuge tube, PBS

4 Protocol of Use

4.1 Day I: Seeding of CTLL-2 instaCELLs and addition of samples

4.1.1 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 or DMEM).

- 12 different dilutions of the IL-2 reference standard are required.
- Dilute the IL-2 reference standard 10 fold in assay buffer L to obtain a 1µg/ml IL-2 stock solution.
- Add 200µl assay buffer L to the first micro tube and add 50µl of the IL-2 stock solution to obtain C1.
- Prepare the remaining eleven micro tubes with 125µl assay buffer L and start a 2-fold dilution series by adding 125µl from C1 in the second tube C2 Proceed until C12.

4.1.2 Thawing and seeding of CTLL2-Cells

- Equilibrate assay buffer to 37°C.
- Thaw one vial of assay ready CTLL-2 cells in a water bath at 37°C for 2min.
- Prepare 4ml of recovery buffer L in a 15ml centrifugation tube. Dispense the cells completely into the prepared tube. Rinse the cryovial once with 1ml recovery buffer.
- Incubate the cells for 2 minutes
- Centrifuge for 5min at 500xg and carefully aspirate the supernatant. Resuspend the cell pellet in 10.8ml of assay buffer L.
- Dispense 90µl of the cell suspension into each well of the provided assay plate, except the wells in row A. Mix cell suspension before each pipetting step.
- Add 90µl assay buffer to each well.
- Pipette 10µl of the IL-2 reference standard dilution series with a 100µl multipipette in dispending mode, into each corresponding well in triplicates of the rows A-D (see plate layout). *Note: Reorder the micro tubes, so that the lowest concentrations are on the outside.*
- Shake the plate for approximately 30 sec. on an orbital plate shaker at 1400 rpm.
- Incubate for 48h in a humidified incubator at 37°C and 5% CO_2.

4.2 Day III: Readout by Resazurin Viability Assay

- Equilibrate the resazurin solution to room temperature.
- If possible, check the cell viability of the cells by morphology and confluence under a microscope
- Add 20µl of Resazurin solution to each well of the assay plate. Shake the plate for 15s and incubate it for 4h at 37°C and 5% CO_2.
- The fluorescence is measured at 540nm and 590nm for reference.

5 Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	~~no cells C12~~	~~no cells C10~~	~~no cells C8~~	~~no cells C6~~	~~no cells C4~~	~~no cells C2~~	~~no cells C1~~	~~no cells C3~~	~~no cells C5~~	~~no cells C7~~	~~no cells C9~~	~~no cells C11~~
B	Ref C12	Ref C10	Ref C8	Ref C6	Ref C4	Ref C2	Ref C1	Ref C3	Ref C5	Ref C7	Ref C9	Ref C11
C	Ref C12	Ref C10	Ref C8	Ref C6	Ref C4	Ref C2	Ref C1	Ref C3	Ref C5	Ref C7	Ref C9	Ref C11
D	Ref C12	Ref C10	Ref C8	Ref C6	Ref C4	Ref C2	Ref C1	Ref C3	Ref C5	Ref C7	Ref C9	Ref C11
E	sample C12	sample C10	sample C8	sample C6	sample C4	sample C2	sample C1	sample C3	sample C5	sample C7	sample C9	sample C11
F	sample C12	sample C10	sample C8	sample C6	sample C4	sample C2	sample C1	sample C3	sample C5	sample C7	sample C9	sample C11
G	sample C12	sample C10	sample C8	sample C6	sample C4	sample C2	sample C1	sample C3	sample C5	sample C7	sample C9	sample C11
H	solvent control											

test sample (triplicates of 12 dilutions), reference standard (triplicates of 12 dilutions), reference standard no cells
solvent control

6 Analysis and Assay Acceptance Criteria

6.1 Calculation of Viability and EC_{50}

If the basis signal from the wells without cells are in the same range, calculate the mean and subtract it from every other well to normalize the raw data and remove the baseline.

Calculate the viability of every concentration for the reference standard. Therefore, calculate the mean of the "Cell only control" and subtract this value for each concentration. Calculate then the viability of the CTLL-2 measured values with equation 1 below:

$$\frac{Mean\ of\ measured\ value\ from\ Substance}{Mean\ of\ highest\ concentration\ from\ Substance}\ x\ 100 = Viability\ [\%]$$

Equation 1: Calculation of the cell viability

Calculate the EC_{50} with a four-parameter fit model, the X axis contains the concentration of the IL-2 and the Y axis the calculated viability. To do so, the GraphPad Prism "Analyze" ➢ "XY analyses" ➢ "Nonlinear regression (curve fit)" shall be used.

6.2 Acceptance Criteria

- The standard deviation of the triplicates must be <20%.
- The EC_{50} of the standard curve must be in a range of 0.5 ng/ml – 1.2 ng/ml.
- The regression of dose-response shall be statistically significant ($p<0.01$).

For results outside these limits, the assay must be repeated.

7 Stability & Storage

Performance of the kit is guaranteed within the specifications as defined in the certificate of analysis only before the expiration date as indicated on the packaging and only if stored and handled according to the instruction of this datasheet.

- Store Assay Ready Cells below -140°C (e.g., in vapor phase of liquid nitrogen).
- Store all other reagents and media as indicated on the label.

8 Literature & Related Documents

[1] Wadhwa M, Bird C, Heath AB, Dilger P, Thorpe R; Participants of the collaborative study. The 2nd International Standard for Interleukin-2 (IL-2). Report of a collaborative study. J Immunol Methods. 2013 Nov 29;397(1-2):1-7. doi: 10.1016/j.jim.2013.07.012. Epub 2013 Aug 13. PMID: 23948423.

[2] SF350-01 IL-2 potency assay kit

9 Support

https://www.accellerate.me/support/contact.html

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10 Disclaimer

The product is sold under the terms of a Limited Use Label License attached to the product. By breaking the seal of the product package, the user explicitly agrees to the license terms. Assay Ready Cells are for immediate assay use only. The user shall not propagate, passage, or refreeze the cells.

This product is intended for research use only. Do not use for diagnostic or therapeutic purposes.