instaCELL TNF-α potency assay kit

Product Information

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Content

1 Description

Tumor necrosis factor alpha (TNF-α) is a multifunctional signaling substance involved in local and systemic inflammation. Its primary function is the regulation of various immune cells and is therefore mainly secreted by macrophages. TNF-α can induce fever, apoptotic cell death, cachexia, and inflammation. A Dysregulation of TNF-α in the human body is associated with diseases like Alzheimer's disease[1], cancer[2], major depression[3], psoriasis[4] and inflammatory bowel disease (IBD)[5].

Figure 1: instaCELL TNF-α potency assay kit

The instaCELL TNF-α potency assay kit is an *in vitro* test to determine the potency of TNF-α for quality control. The assay utilizes the cytotoxic effect of TNF-α on the well-established mouse fibroblast cell line L-929. The assay kit uses a Resazurin viability assay to analyze the potency of the TNF-α product and compares it to the international standard of the NIBSC.

The instaCELL TNF-α potency assay kit is based on the WHO publication "Report on a Collaborative study for proposed 3rd International standard for Tumor Necrosis Factor alpha (TNF-α)"[6]. It includes prequalified Assay Ready L-929 Cells as well as media, reagents, international TNF-α standard control, and 96-well plate to perform the assay according to the WHO. Assay Ready Cells are frozen cell aliquots which can be used directly in the assay without prior cultivation, basically like a reagent.

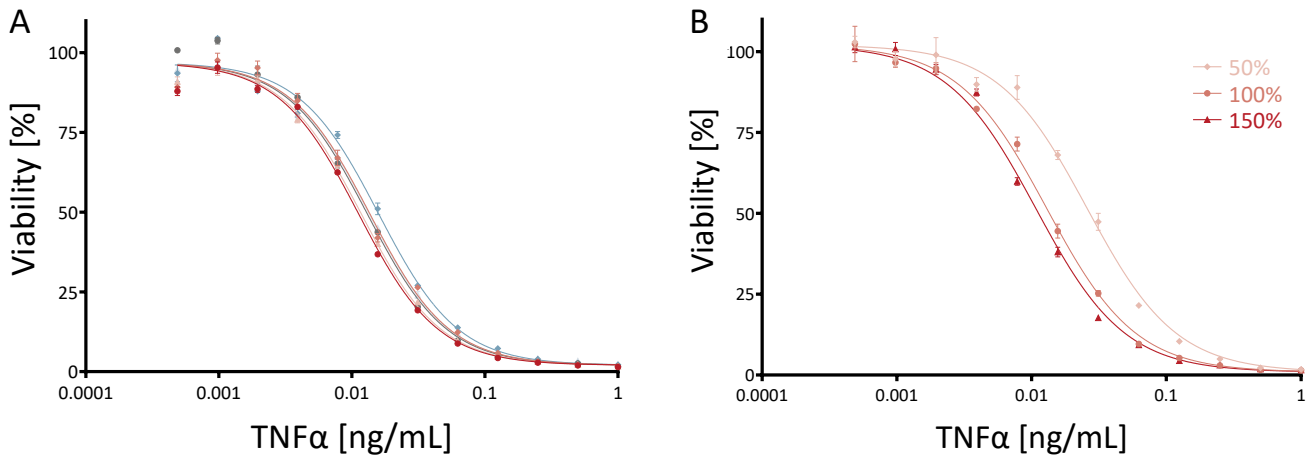

Figure 2: Precision & Accuracy of the instaCELL TNF-α potency assay kit

[A] The cytotoxic potency of a given TNF-α preparation can be precisely determined with L-929 Assay Ready Cells. In six independent experiments performed at different days using six different aliquots of ARCs an average EC_{50} of 0.013 ng/ml was determined at a CV of 12% (R^2=0.997)

[B] The accuracy of ARCs was confirmed in a spiking experiment where nominal concentrations of 50%, 100% and 150% of TNF-α were applied and potencies of 49%, 100% and 141% were determined, which is an average accuracy of 95.5%.

2 Cell Information

Source:	Cell Line Service GmbH
Cell Type:	Fibroblast
Tissue:	Connective Tissue
Species:	Mouse
Growth:	Adherent
Gender:	Male
Biosafety Level:	1

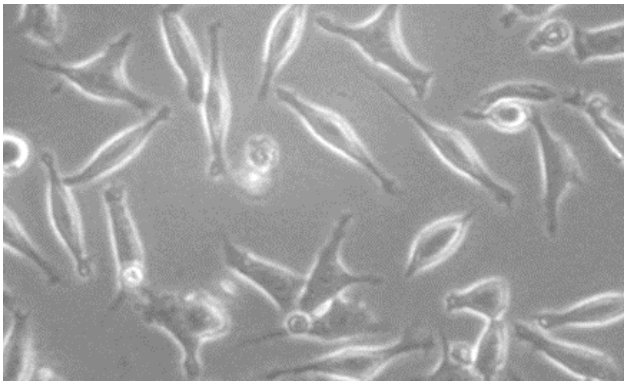

Figure 3: Morphology of Assay Ready L-929 Cells

3 Kit Content

• L-929 assay ready cells	1 vial (3E+06 cells)	RE772K
• Recovery Buffer A	1 bottle (10ml)	MD163-01
• Assay Buffer A	2 bottle (60ml)	MD363-06
• TNF-α Stock (2 µg/ml)	1 vial (25µl)	RX517-01
• Actinomycin D	1 vial (1mg)	10710.01 (Serva)
• Resazurin	1 bottle (5ml)	RX718-01
• 96-well plate	1 plate	83.3924.300 (Sarstedt)

Additionally required but not provided with the kit:

DMSO

4 Protocol of Use

4.1 Day I: Seeding of L-929 Cells

- Keep the cells on dry ice before thawing and process quickly.
- Equilibrate all media and buffer to 37°C.
- Thaw the Assay Ready Cells in a water bath at 37°C for 3min. Prepare 8ml of recovery buffer in a 50ml centrifuge tube. Dispense the cells completely into the prepared tube. Rinse the cryovial once with 1ml recovery buffer.
- Centrifuge for 3min at 200xg and carefully aspirate the supernatant, flick the centrifuge tube to loosen the cell pellet. Resuspend the pellet in 30ml of assay buffer. Mix cell suspension before each pipetting step. Dispense 150µl of the cell suspension into each well of the provided assay plate, except the wells in row A (see plate layout).
- Incubate for 24h in a humidified incubator at 37°C and 5% CO_2.

4.2 Day II: Preparation and addition of test sample and controls to the cells

Caution: Actinomycin is sensitive to light, switch off the light of the sterile hood!

4.2.1 Preparation of the working medium:

- Dissolve Actinomycin D in 1ml DMSO, mix well. Dilute the solution 10-fold in assay buffer (50µl + 450µl).
- Dilute the solution again 100-fold (270µl+26.73ml) in assay buffer to obtain the working medium (1µg/ml).

4.2.2 Preparation of the TNF-α working solution:

- Dilute the TNF-α stock solution **two times** 10-fold in working medium (10µl + 90µl).
- Further dilute 70µl of dilution 2 in 1330µl working medium to obtain the top concentration of the TNF-α standard curve (1ng/ml).
- Perform a 2-fold serial dilution series of 12 concentrations for the TNF-α standard curve. Add 700µl of C1 to 700µl of working medium, mix well and continue this process up to C12.
- Prepare a dilution series of your test sample analogue to the TNF- α standard dilution series in working medium.
- Check the cell viability and confluence under a microscope.
- Aspirate the medium from the assay plate and add 150µl of the dilution series of TNF-α standard (rows A-D) and test sample (rows F-H) to the corresponding wells. To avoid any edge effect, use the inverse concentration order from the plate layout below (Fig. 4).
- Add 150µl of working medium to the wells E1-E6 for Actinomycin D control and 150µl of assay buffer to the wells E7-E12 for medium control. Shake the plate for 15s on an orbital shaker.
- Incubate for 24h in a humidified incubator at 37°C and 5% CO_2.

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

Figure 4: Recommended plate layout

test sample (triplicates of 12 dilutions), TNF-α STD (triplicates of 12 dilutions), Actinomycin D control (sextet), medium control (sextet)

4.3 Day III: Readout by Resazurin Viability Assay

- Equilibrate Resazurin Solution to room temperature.
- Add 20µl of Resazurin Solution to each well of the assay plate. Shake the plate for 15s on an orbital shaker.
- Incubate for 4h in a humidified incubator at 37°C and 5% CO_2.
- Shake the plate for 15s on an orbital shaker. Afterwards measure the fluorescence at $540_{EX}/590_{EM}$.

5 Analysis and Assay Acceptance Criteria

5.1 Calculation of Viability and EC_{50}

- Calculate the mean of the wells of row A and subtract it from every other well for baseline correction.
- Calculate the mean of the control sextets.
- Calculate the viability of every concentration for the test sample and the reference standard with the following equation:

$$\frac{\text{Mean of triplicate}}{\text{Mean of Actinomycin D control}} \text{ x } 100 = \text{Viability [\%]}$$

Equation 1: Calculation of the cell viability.

- Calculate the EC50 with a four-parameter fit model, the X axis contains the concentration of the TNF-α and the Y axis the calculated viability.

5.2 Acceptance Criteria

- The criterion that must be met is the parallelism of the dose-response curves. For this, the "slope ratio" must be in the range of 0.67 to 1.5. If this is not the case, the values are outside the WHO specification, and the assay is invalid.

$$Slope\ Ratio = \frac{Slope_{sample}}{Slope_{Ref}}$$

Equation 2: Calculation of the Slope Ratio to check for parallelism.

- The potency achieved by the TNF-α test must not be less than 80% and no more than 120% of the reference standard.
- The confidence limits (P = 0.95) must not be below 80% and not above 125%.
- The range of the EC_{50} of the TNF-α standard curve should be between 6 pg/ml and 21 pg/ml.

If the value is outside of these limits, the assay must be repeated.

6 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.

7 Literature & Related Documents

[1] Swardfager W, Lanctôt K, Rothenburg L, Wong A, Cappell J, Herrmann N (2010). "A meta-analysis of cytokines in Alzheimer's disease". Biol Psychiatry. 68 (10): 930–941. PMID 20692646

[2] Locksley RM, Killeen N, Lenardo MJ (2001). "The TNF and TNF receptor superfamilies: integrating mammalian biology". Cell. 104 (4): 487–501. PMID 11239407.

[3] Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, Lanctôt KL (2010). "A meta-analysis of cytokines in major depression". Biol Psychiatry. 67 (5): 446–457. PMID 20015486.

[4] Victor FC, Gottlieb AB (2002). "TNF-alpha and apoptosis: implications for the pathogenesis and treatment of psoriasis". J Drugs Dermatol. 1 (3): 264–75. PMID 12851985

[5] Brynskov J, Foegh P, Pedersen G, Ellervik C, Kirkegaard T, Bingham A, Saermark T (2002). "Tumour necrosis factor alpha converting enzyme (TACE) activity in the colonic mucosa of patients with inflammatory bowel disease". Gut. 51 (1): 37–43. PMID 12077089

[6] Wadhwa, Meenu, Bird, Chris, Dilger, Paula, Hockley, Jason, Rigsby, Peter. et al. (2013). Report on a collaborative study for proposed 3rd international standard for tumor necrosis factor -alpha (TNF-α). World Health Organization.

8 Support

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

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9 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.