Quantification of functional TNF-alpha using *iLite*® TNF-alpha Xcel Assay Ready Cells

For research and professional use only. Not for use in diagnostic procedures.

This application note contains a suggested protocol and performance data.
Each individual laboratory must set up their own method and perform relevant validations.

Background

TNF-alpha promotes inflammatory responses, which in turn contribute to the clinical symptoms associated with many inflammatory disorders, including rheumatoid arthritis, ankylosing spondylitis, Crohn's disease, psoriasis and refractory asthma. (1) These diseases are in many cases treated with TNF-alpha inhibitors, such as **infliximab, adalimumab, etanercept, golimumab,** and **certolizumab pegol**. Prolonged therapies with these TNF-alpha inhibitors may lead to the development of neutralizing antibodies (NAbs), which may counteract the TNF-alpha antagonist activity of the inhibitors. (2)

The *iLite*® TNF-alpha Xcel Assay Ready Cells can be used for measurements of functional TNF-alpha, TNF-alpha inhibitor activity and presence of neutralizing antibodies to TNF-alpha inhibitors.

Principle of the assay

The *iLite*® TNF-alpha Xcel Assay Ready Cells are engineered cells, optimized to be responsive to TNF-alpha, resulting in a proportional expression of Firefly Luciferase (FL). Binding of TNF-alpha to its receptor results in activation of the NFkB regulated Firefly luciferase reporter gene construct. *iLite*® TNF-alpha Xcel Assay Ready Cells also contain the Renilla Luciferase (RL) reporter gene, under the control of a constitutive promoter. The constitutive expression of RL allows normalization of TNF-alpha induced FL activity, and renders assay results independent of variations in cell number or serum matrix effects. The luciferase signal can be measured in a luminometer following addition and incubation of luciferase substrate. The Firefly luciferase signal is proportional to the concentration of functional TNF-alpha in a sample (Fig.1).

Specimen collection

The *iLite*® TNF-alpha Xcel Assay Ready Cells can be used for measuring concentration of TNF-alpha in test samples including human serum.

Material and equipment needed

Material and equipment	Suggested supplier	Reference
iLite® TNF-alpha Xcel Assay Ready Cells	Svar Life Science	BM4044
Diluent (DMEM containing 9% heat inactivated FBS + 1% Penicillin-Streptomycin)	Gibco	31966-021 (DMEM) 26140-079 (FBS) 15140-122 (Penicillin-Streptomycin)
TNF-alpha or analogues	R&D Systems	210-TA/CF
Firefly/Renilla luciferase substrate	Promega	E2920, Dual-Glo Luciferase Assay System
Plate; White walled micro well plate suitable for luminescence	Revvity	6055680

Microplate Luminometer with appropriate reading software – no filter on luminometer	Contact Svar Life Science for list of recommended suppliers	NA
Incubator, 37 °C with 5% CO_2	NA	NA
Water bath, 37 °C	NA	NA
Single-channel and multi-channel pipettes with polypropylene disposable tips	NA	NA
Polypropylene tubes or plate for dilution	NA	NA
Single-use polypropylene reservoir	NA	NA
Plate shaker	NA	NA
Timer	NA	NA

Protocol

Preparation of calibrators (TNF-alpha)

Recombinant TNF-alpha from R&D Systems has been used to stimulate the *iLite*® TNF-alpha Xcel Assay Ready Cells. The below table shows the dilutions of TNF-alpha, used for QC release of the *iLite*® TNF-alpha Xcel Assay Ready Cells.

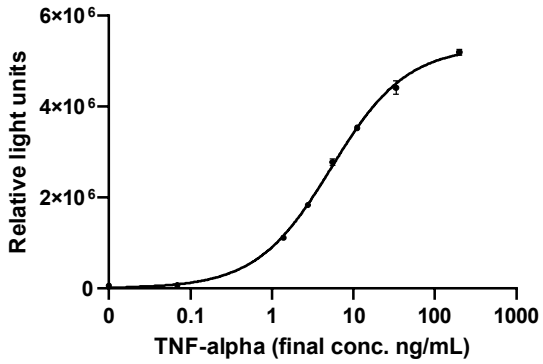

Figure 1. Example of TNF-alpha calibration curve.

Calibrator	TNF-alpha Suggested calibrator solution conc. (ng/mL)
A	400
B	67
C	22
D	11
E	5.6
F	2.8
G	0.14
H	0

Table 1. Suggested calibrator **solution concentrations** for TNF-alpha.

Assay preparation and incubation

1. Design a plate layout. It is recommended to perform the test at least in duplicates.
2. Dilute calibrators, controls, and samples to fall within the expected in assay values of 0 - 200 ng/mL.
3. Add 40 µL calibrators, controls, and samples in duplicate to assigned wells (final concentration will be half of solution concentration).
4. Thaw the vial of *iLite*® TNF-alpha Xcel Assay Ready Cells in a 37°C water bath with gentle agitation. The cell suspension is mixed very carefully ten times with pipette to ensure a homogeneous distribution of cells.
5. Dilute 250 µL cell suspension with 5.75 mL Diluent.
6. Add 40 µL diluted cells to each well.
7. Place the lid on the plate, mix and incubate for 4 hours at 37 °C with 5% CO_2.

Adding substrate solutions

8. Equilibrate the plate and the substrate solution to room temperature.
9. Prepare the **Firefly luciferase** substrate according to the supplier's instructions and add 80 µL per well. Mix and protect the plate from light. After 10 minutes incubation at room temperature read in a luminometer.

10. If appropriate, prepare the **Renilla luciferase** substrate according to the supplier's instructions and add 80 µL per well. Mix and protect the plate from light. After 10 minutes incubation at room temperature read in a luminometer.

Precautions

- This application note is intended for professional laboratory research use only. The data and results originating from following the Application Note should not be used either in diagnostic procedures or in human therapeutic applications.
- Use and handle the material and instruments referenced according to the supplier's/manufacturer's instructions or product specifications accompanying the individual material and instruments.
- Dispose of all sample specimens, infected or potentially infected material in accordance with good microbiological practice. All such materials should be handled and disposed as potentially infectious.
- Residues of chemicals and preparations are generally considered as biohazardous waste and should be inactivated prior to disposal by autoclaving or using bleach. All such materials should be disposed in accordance with established safety procedures.

Proprietary Information

In accepting delivery of *iLite*® Assay Ready Cells the recipient agrees not to sub-culture these cells, attempt to sub-culture them or to give them to a third-party recipient, and only to use them directly in assays. *iLite*® cell-based products are covered by patents which are the property of Svar Life Science AB and any attempt to reproduce the delivered *iLite*® Assay Ready Cells is an infringement of these patents.

QUICK GUIDE

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1 Sample dilution

- Equilibrate reagents and samples to room temperature – **do not thaw cells and substrate reagents at this stage**
- Dilute calibrators, controls and samples.
- Add 40 µL of calibrators, controls and diluted samples to pre-assigned wells.
- Thaw the cell vial in a 37°C water bath. Dilute the cells. Invert the cell suspension in order to ensure a homogeneous cell solution.
- Add 40 µL diluted cells to each well.

2 Incubation 4 h

- Incubate at 37 °C with 5% CO_2 for 4 hours.

3 Read plate

- Equilibrate the plate to room temperature
- Prepare the **Firefly luciferase** substrate according to the supplier's instructions and add 80 µL per well. Mix. Protect the plate from light. After 10 min incubation read in a luminometer.
- If appropriate, prepare the **Renilla luciferase** substrate according to the supplier's instructions and add 80 µL per well. Mix. Protect the plate from light. After 10 min incubation read in a luminometer.

Troubleshooting and FAQ

Please consult the Svar Life Science website www.svarlifescience.com

References

1. Kalliolias GD, Ivashkiv LB. *TNF biology, pathogenic mechanisms and emerging therapeutic strategies.* Nat Rev Rheumatol. 2016 Jan;12(1):49-62.
2. Kalden JR, Schulze-Koops H. *Immunogenicity and loss of response to TNF inhibitors: implications for rheumatoid arthritis treatment.* Nat Rev Rheumatol. 2017 Nov 21;13(12):707-718.

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