

Quantification of functional TNF-alpha using *iLite*[®] TNF-alpha 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**, or **etanercept** to mention a few. Prolonged therapies with these TNF-alpha inhibitors may lead to development of neutralizing antibodies (NABs), which may counteract the TNF-alpha antagonist activity of the inhibitors. (2)

The *iLite*[®] TNF-alpha 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. (3,4)

Principle of the assay

The *iLite*[®] TNF-alpha Assay Ready Cells are engineered cells, optimized to express Firefly luciferase (FL) under the control of a NFκB responsive promoter. Binding of TNF-alpha to its receptor results in activation of the NFκB regulated Firefly luciferase reporter gene construct. *iLite*[®] TNF-alpha 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 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
<i>iLite</i> [®] TNF-alpha Assay Ready Cells	Svar Life Science	BM3044
Diluent (RPMI containing 9% heat inactivated FBS + 1% Penicillin-Streptomycin)	Gibco	61870-044 (RPMI) 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	PerkinElmer	6005680
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 Assay Ready Cells. The below table shows the dilutions of TNF-alpha, used for QC release of the *iLite*[®] TNF-alpha Assay Ready Cells.

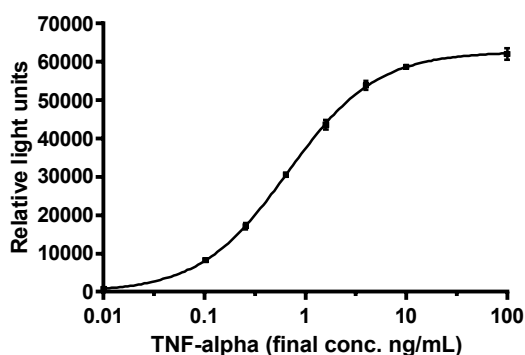


Figure 1. Example of TNF-alpha calibration curve.

Calibrator	TNF-alpha
	Suggested calibrator solution conc. (ng/mL)
A	200
B	20
C	8.0
D	3.2
E	1.3
F	0.51
G	0.20
H	0

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

Incubation

1. Design a plate layout. It is recommended to perform the test at minimum in duplicates.
2. Dilute calibrators, controls and samples to fall within the expected **in assay values** of 0-100 ng/mL.
3. Add 50 μ L calibrators, controls and samples in duplicates to assigned wells (final concentration will be half of the solution concentration).
4. Thaw a vial of *iLite*[®] TNF-alpha Assay Ready Cells in a 37 °C water bath with gentle agitation.
5. Add the entire content of the cell vial to 6 mL Diluent. Invert the vial containing the diluted cell suspension approximately ten times to ensure a homogeneous distribution of cells.
6. Add 50 μ L diluted cells to each well.
7. Place the lid on the plate, mix and incubate for 3 hours at 37 °C with 5% CO₂.

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 μ L 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

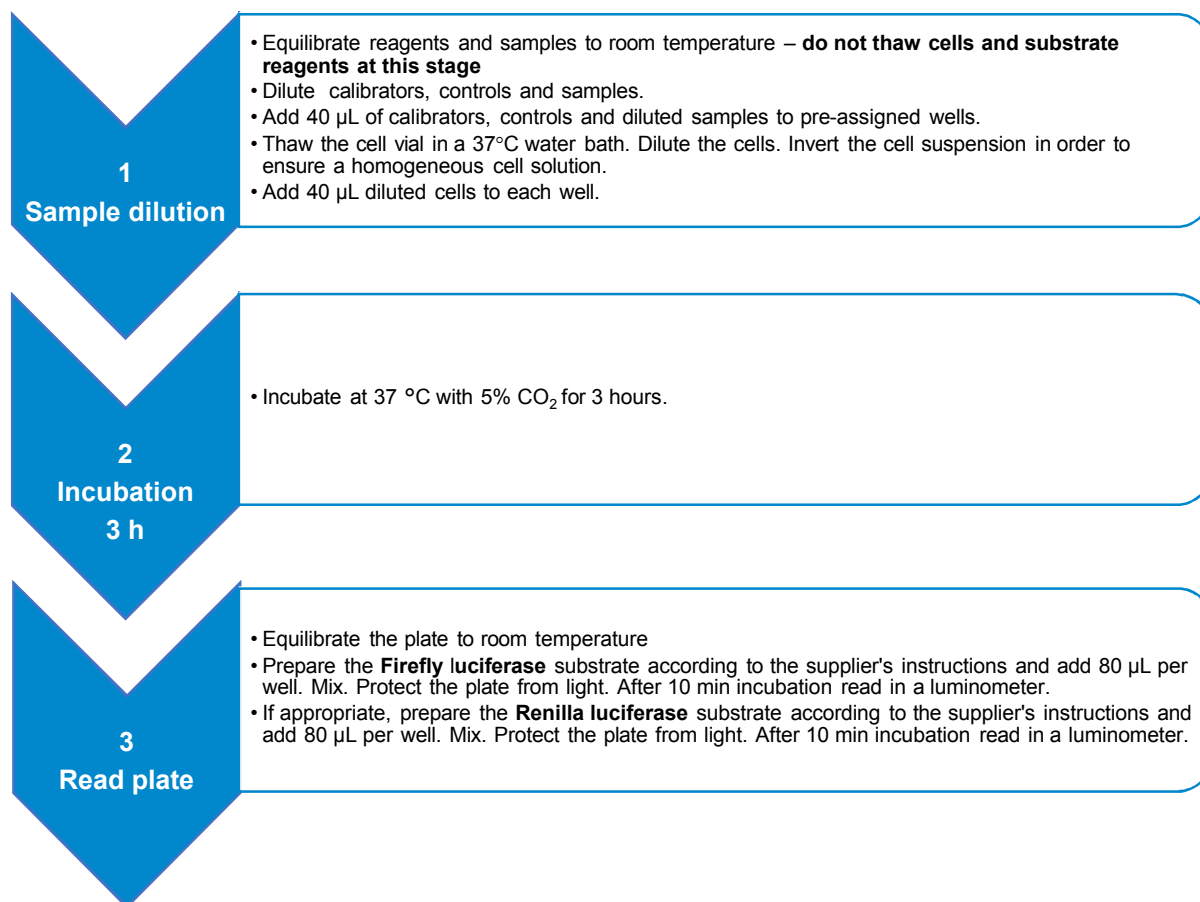
- This application note is intended for professional laboratory research use only. The data and results originating from following the Application Note should not be used either in diagnostic procedures or in human therapeutic applications.
- Use and handle the material and instruments referenced according to the supplier's/manufacture'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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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.
3. Lallemand C, Tovey MG. et al. *Reporter gene assay for the quantification of the activity and neutralizing antibody response to TNF-alpha antagonists*. J Immunol Meth. 2011, 373: 229-239.
4. Pavlov I, Delgado JC et al. *Clinical laboratory application of a reporter-gene assay for measurement of functional activity and neutralizing antibody response to infliximab*. Clinica Chimica Acta. 2016, 453:147-153.