Quantification of LPS using *iLite*® TLR4 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

The toll-like receptor (TLR) family consist of receptors responsible for pattern recognition in innate immunity, key in the detection of pathogens and immune responses (1). The TLR4 is the most studied member of this family and induces pro-inflammatory responses upon invasion of pathogens. TLR4 is activated by binding of lipopolysaccharide (LPS, endotoxin) from Gram-negative bacteria (2). An important role of TLR4 is described in many inflammatory diseases including sepsis, asthma, cancer, acute kidney injury, or intestinal inflammation among others (1–4). Briefly, TLR4 signalling is induced upon activation in the plasmatic membrane. The signal transduction extends through TIRAP and MyD88 adaptor proteins, in early endosomes the signal pathway continues via the adaptor proteins TRAM and TRIF (2). Currently, scientist attention had been drawn to identify new molecules that can inhibit/reduce TLR4 signalling for several diseases (1,3,4).

Principle of the assay

The *iLite*® TLR4 Assay Ready Cells are engineered cells optimized to express Firefly luciferase under the control of a NFKB responsive promoter. Binding of LPS to membrane bound Toll-like Receptor 4 (TLR4) results in activation of the NFKB responsive Firefly luciferase reporter gene construct. *iLite*® TLR4 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 LPS induced FL activity, and renders assay results independent of 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 activity of LPS in a sample (Fig.1).

Specimen collection

The *iLite*® TLR4 Assay Ready Cells can be used for measuring activity of LPS in test samples including human serum.

Svar Life Science AB
Lundavägen 151
SE-212 24 Malmö
Sweden

P.O. Box 50117
SE-202 11 Malmö
Sweden

info@svarlifescience.com
+46 40 53 76 00

Material and equipment needed

Material and equipment	Suggested supplier	Reference
iLite® TLR4 Assay Ready Cells	Svar Life Science	BM4025
Diluent (RPMI containing 9% heat inactivated FBS + 1% Penicillin-Streptomycin)	Gibco	61870-044 (RPMI) 26140-079 (FBS) 15140-122 (Penicillin-Streptomycin)
LPS	Invivogen	Tlrl-3pelps
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 (LPS)

Ultrapure LPS (E.coli 0111:B4) from Invivogen has successfully been used to stimulate the *iLite*® TLR4 Assay Ready Cells. The below table shows the dilutions of LPS, used for QC release of the *iLite*® TLP4 Assay Ready Cells.

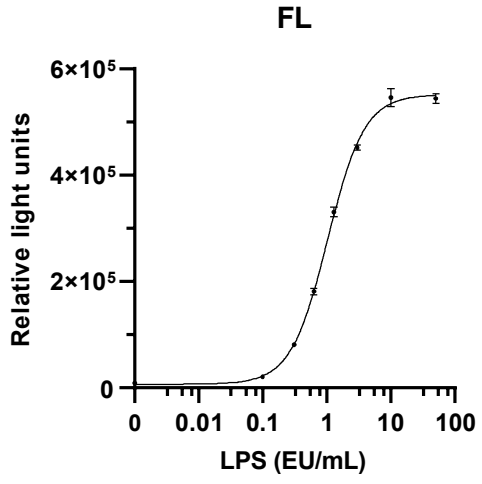


Figure 1. Example of LPS calibration curve.

Calibrator	LPS Suggested calibrator solution conc. (EU/ml)
A	100
B	20
C	5.0
D	2.5
E	1.3
F	0.63
G	0.21
H	0

Table 1. Suggested calibrator **solution concentrations** for LPS.

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 0-50 EU/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*® TLR4 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 5 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 suppliers 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 suppliers 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

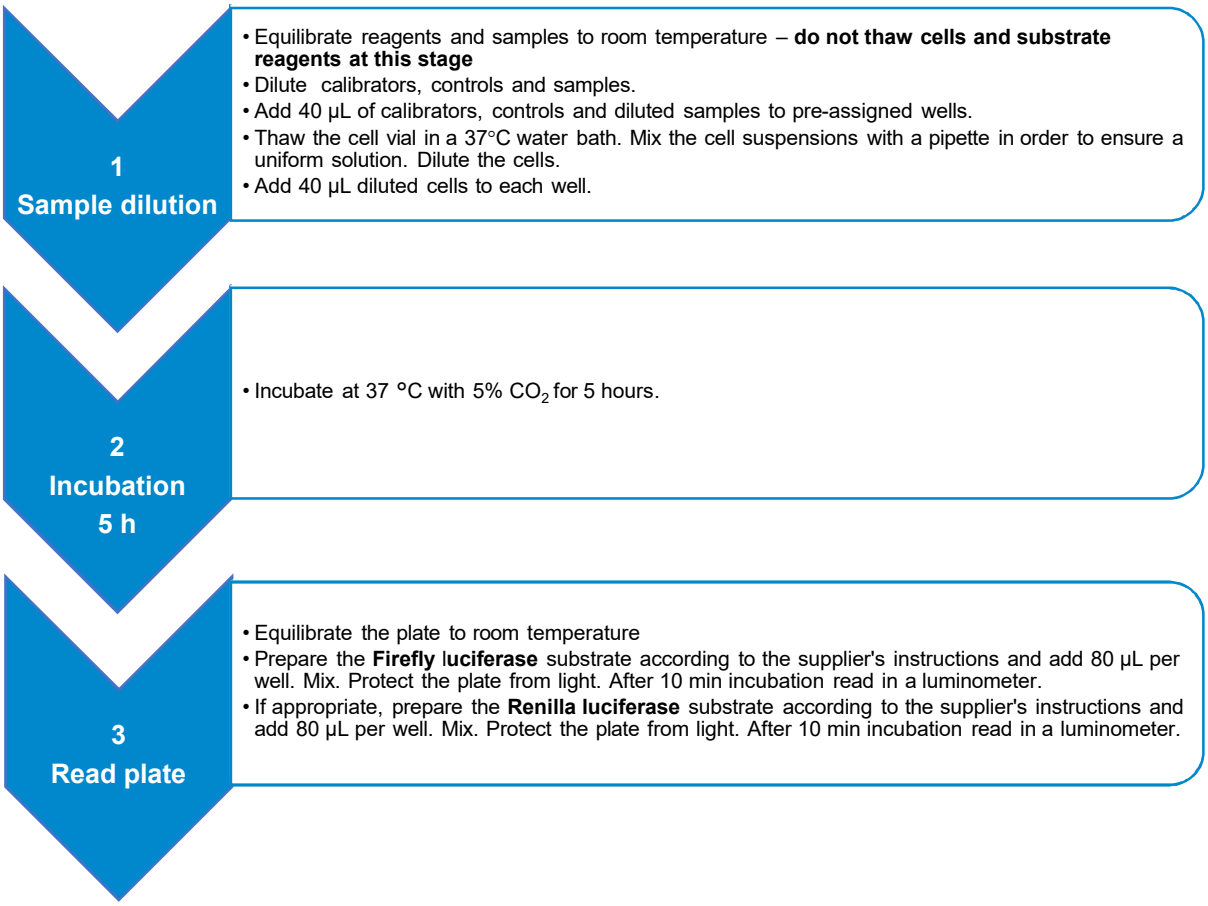
- This application note is intended for professional laboratory research use only. The data and results originating from following the Application Note should not be used either in diagnostic procedures or in human therapeutic applications.
- Use and handle the material and instruments referenced according 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 though 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 of 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

Quantification of LPS using *iLite*® TLR4 Assay Ready Cells



Troubleshooting and FAQ

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

References

1. Kashani B, Zandi Z, Pourbagheri-Sigaroodi A, Bashash D, Ghaffari SH. The role of toll-like receptor 4 (TLR4) in cancer progression: A possible therapeutic target? J Cell Physiol. 2021;236(6):4121–37.

2. Ciesielska A, Matyjek M, Kwiatkowska K. TLR4 and CD14 trafficking and its influence on LPS-induced pro-inflammatory signaling. Cell Mol Life Sci. 2021;78(4):1233–61.

3. Jha AK, Gairola S, Kundu S, Doye P, Syed AM, Ram C, et al. Toll-like receptor 4: An attractive therapeutic target for acute kidney injury. Life Sci. 2021;271:119155.

4. Tam JSY, Coller JK, Hughes PA, Prestidge CA, Bowen JM. Toll-like receptor 4 (TLR4) antagonists as potential therapeutics for intestinal inflammation. Indian J Gastroenterology. 2021;1–17