

Quantification of IL-6 receptor inhibitor using *iLite*[®] IL-6 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

Interleukin-6 (IL-6) is member of the cytokine family. It is found relevant to many inflammatory diseases and many cancers and can act as both a pro-inflammatory cytokine and an anti-inflammatory myokine. In classic signaling, IL-6 stimulates target cells via a membrane bound interleukin-6 receptor (IL-6R), which upon ligand binding associates with the signaling receptor protein gp130. The anti-inflammatory activities of IL-6 are transmitted by this signaling pathway whereas pro-inflammatory responses of IL-6 are mediated by trans-signaling (cells expressing only gp130 respond to a complex of IL-6 bound to a soluble form of the IL-6R). This is important since therapeutic inhibition of IL-6 by the neutralizing anti-IL-6R monoclonal antibody has been focused on the treatment of inflammatory diseases (1). One of these therapeutic antibodies is tocilizumab, an immunosuppressive drug, mainly used for the treatment of rheumatoid arthritis and systemic juvenile idiopathic arthritis. Tocilizumab is a humanized monoclonal antibody against the IL-6R and functions as an IL-6 inhibitor that binds to soluble as well as membrane bound IL-6R, preventing IL-6 from exerting its pro-inflammatory effects (2).

Principle of the assay

The *iLite*[®] IL-6 Assay Ready Cells are engineered cells optimized to express Firefly luciferase under the control of a IL-6 responsive promoter. Binding of IL-6 to the membrane bound IL-6R (IL-6RA and IL6ST) results in activation of the IL-6 regulated Firefly luciferase reporter gene construct. *iLite*[®] IL-6 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 IL-6 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 functional activity of IL-6 in the sample. In the presence of inhibitory activity against the IL-6R, the functional activity of the present IL-6 is reduced, resulting in a decreased stimulation of Firefly luciferase production.

Thus, the Firefly luciferase signal is inversely proportional to the amount of inhibitory activity against IL-6R in a sample. The *iLite*[®] IL-6 Assay Ready Cells can therefore be utilized as a highly sensitive assay for quantification of IL-6R inhibitor activity in test samples, including human serum.

Material and equipment needed

Material and equipment	Suggested supplier	Reference
<i>iLite</i> [®] IL-6 Assay Ready Cells	Svar Life Science	BM4061
Diluent (DMEM containing 9% heat inactivated FBS + 1% Penicillin-Streptomycin).	Gibco	31966-021 (DMEM) 26140-079 (FBS) 15140-122 (Penicillin-Streptomycin)
Tocilizumab	NA	NA
IL-6 or analogues	R&D Systems	206-IL
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 IL-6 receptor inhibitor

Tocilizumab from Hoffmann-La Roche has successfully been used to neutralize functional activity of IL-6 and inhibit the IL-6 regulated Firefly luciferase expression in *iLite*[®] IL-6 Assay Ready Cells (refer to the table and graph below).

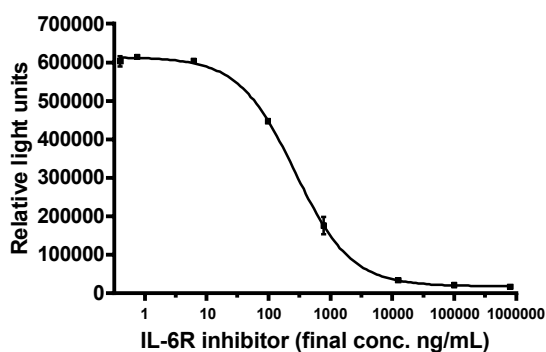


Figure 1. Example of IL-6R inhibitory curve

Final 2000 pg/mL IL-6	Tocilizumab
	Suggested calibrator solution concentrations, ng/mL
A	3 200 000
B	400 000
C	50 000
D	3 125
E	391
F	24
G	3.1
H	0

Table 1. Suggested calibrator solution concentrations for anti-IL6R Tocilizumab

Incubation

1. Design a plate layout. It is recommended to perform the test at least in duplicates.
2. Perform a serial dilution of the reference IL-6R inhibitor. Ensure matrix consistency between reference antibody solutions, control solutions, and sample solutions.
3. Add 20 μL of the reference IL-6R inhibitor dilutions, controls and samples to assigned wells (final concentration will be a quarter of solution concentration).
4. Thaw the vial of *iLite*[®] IL-6 Assay Ready Cells in a 37°C water bath with gentle agitation. The cell suspension is mixed very carefully ten times with pipette in order to ensure a homogeneous distribution of cells.
5. Dilute 250 μL cell suspension with 2.75 mL Diluent.
6. Add 20 μL diluted cells to each well.
7. Place the lid on the plate, mix and incubate the plate for 30 minutes at 37 °C with 5% CO₂
8. Add 40 μL of 4000 pg/ml IL-6 to all wells (final concentration will be 2000 pg/mL IL-6).
9. Place the lid on the plate, mix and incubate for 6 hours at 37 °C with 5% CO₂.

Adding substrate solutions

10. Equilibrate the plate and the substrate solution to room temperature.
11. 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.
12. 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

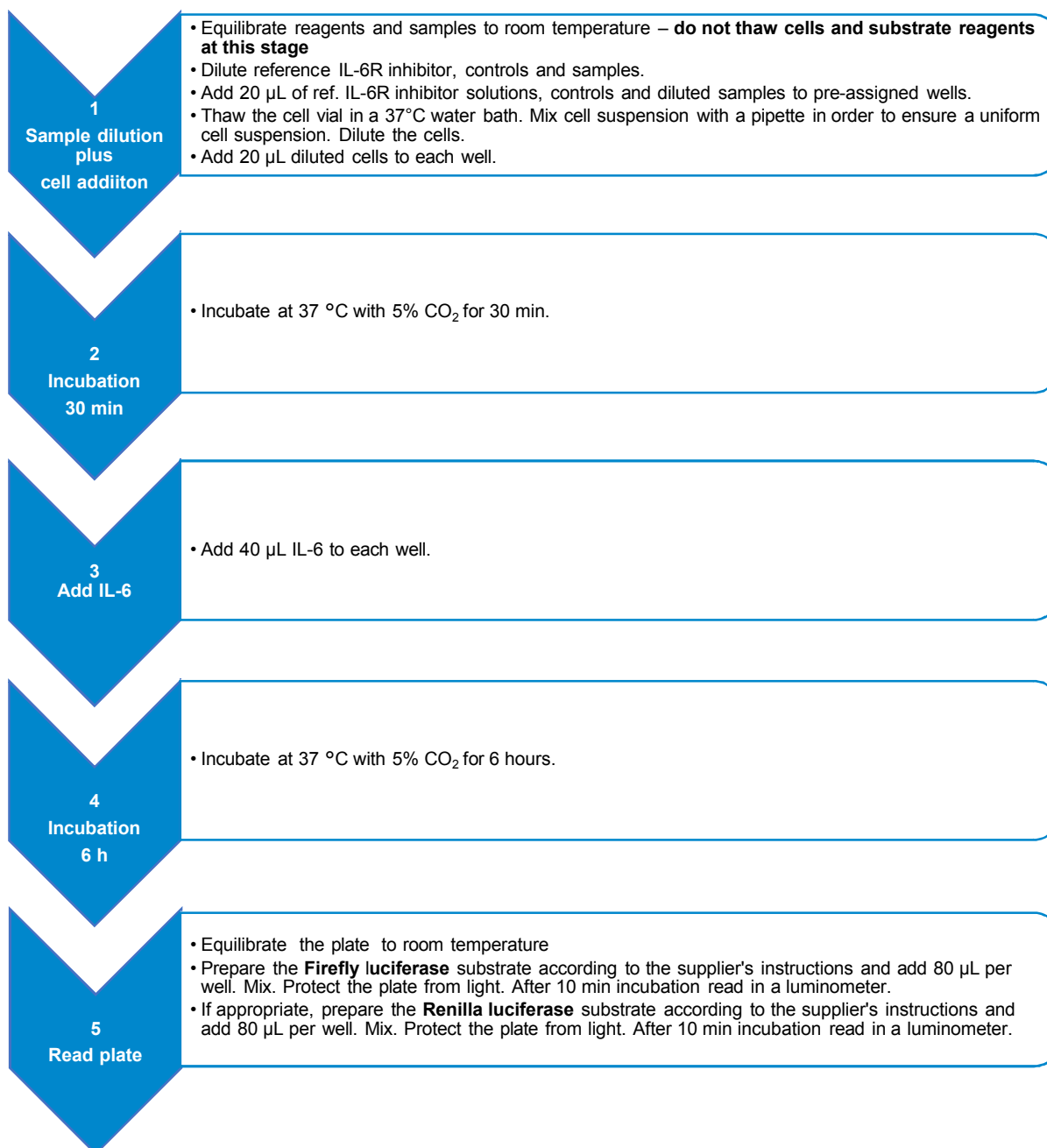
- This application note is intended for professional laboratory research use only. The data and results originating from following the Application Note should not be used either in diagnostic procedures or in human therapeutic applications.
- Use and handle the material and instruments referenced according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

Propriety 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 IL-6R inhibitor activity using *iLite*[®] IL-6 Assay Ready Cells



Troubleshooting and FAQ

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

References

1. Jürgen Scheller, Athena Chalaris, Dirk Schmidt-Arras, Stefan Rose-John, *The pro- and anti-inflammatory properties of the cytokine interleukin-6*. *Biochimica et Biophysica Acta* 1813: 878–888. (2011)
2. Jones, G; Sebba, A; Gu, J; Lowenstein, MB; Calvo, A; Gomez-Reino, JJ; Siri, DA; Tomsic, M; et al, "*Comparison of tocilizumab monotherapy versus methotrexate monotherapy in patients with moderate to severe rheumatoid arthritis: The AMBITION study*". *Annals of the Rheumatic Diseases*. 69 (1): 88–96. (2010)