Quantification of IL-2 inhibitor using *iLite*® IL-2 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-2 (IL-2) is part of the body's natural response to microbial infection and a tool in the immune system discrimination between foreign ("non-self") and "self". It signals through the IL-2 receptor, a complex consisting of three chains, termed alpha, beta, and gamma.
IL-2 has essential roles in key functions of the immune system, tolerance and immunity, primarily as a stimulant of T- and B-cell growth and maturation. IL-2 activates and stimulates the growth of immune cells, most importantly T-Cells, but also Natural Killer Cells (NK Cells), both can eliminate cancer cells directly. Anti-tumor effects of IL-2 appear to be mediated by its effects on NK Cells, Lymphokine-activated Killer Cells and other cytotoxic cells (1,2).
IL-2 is known to increase the number and activity of certain types of white blood cells which is utilized in different cancer treatments. Various pharmaceutical analogues of IL-2 (ex. Aldesleukin) utilize this therapeutic effect. Prolonged therapies with IL-2 analogues may lead to development of neutralizing antibodies (NAbs), which may counteract the IL-2 analog activity. The *iLite*® IL-2 Assay Ready Cells can be used for quantification of IL-2 inhibitory activity.

Principle of the assay

The *iLite*® IL-2 Assay Ready Cells are engineered cells, optimized to express Firefly luciferase (FL) under the control of an IL-2 responsive promoter. Binding of IL-2 to the IL-2 receptor (IL-2Rα, IL-2Rβ, and IL-2Rγ) results in activation of the IL-2 regulated Firefly luciferase reporter gene construct. *iLite*® IL-2 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-2 induced FL activity, and renders assay results independent of variations in cell number or serum matrix effects. The Firefly 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-2 in the sample. In the presence of inhibitory activity against IL-2, the amount of free IL-2 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-2 in a sample. The *iLite*® IL-2 Assay Ready Cells can therefore be utilized as an assay for quantification of IL-2 inhibitor activity in test samples, including human serum.

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Material and equipment needed

Material and equipment	Suggested supplier	Reference
iLite® IL-2 Assay Ready Cells	Svar Life Science	BM4002
Diluent (RPMI containing 9% heat inactivated FBS + 1% Penicillin-Streptomycin).	Gibco	61870-044 (RPMI) 26140-079 (FBS) 15140-122 (Penicillin-Streptomycin)
Rabbit anti-human IL-2 polyclonal antibody	Bio-rad	AHP381Z
IL-2 or analogues	Immunotools	11340025
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 IL-2 inhibitor

IL-2 inhibitor from Bio-rad has successfully been used to neutralize IL-2 and inhibit the IL-2 regulated Firefly luciferase expression in *iLite*® IL-2 Assay Ready Cells (refer to the table and graph below).

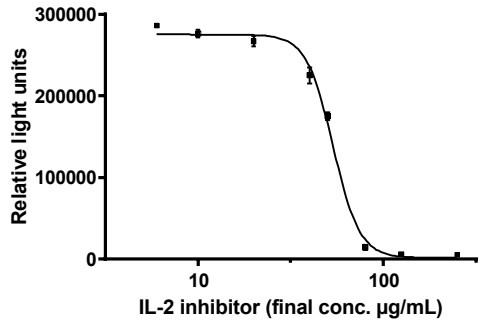


Figure 1. Example of IL-2 inhibitory curve

Final 200 ng/mL IL-2	PAb anti-IL-2 Suggested calibrator solution concentrations, µg/mL
A	250
B	125
C	80
D	50
E	40
F	20
G	10
H	0

Table 1. Suggested calibrator solution concentrations for IL-2 inhibitor

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-2 inhibitor. Ensure matrix consistency between reference antibody solutions, control solutions, and sample solutions.
3. Add 20 µL of the reference IL-2 inhibitor dilutions, controls and samples to assigned wells (final concentration will be a quarter of solution concentration).
4. Add 20 µL of 800 ng/ml IL-2 to all wells (final concentration will be 200 ng/mL IL-2).
5. Place the lid on the plate, mix and incubate the plate for 30 minutes at 37 °C with 5% CO_2
6. Thaw the vial of *iLite*® IL-2 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.
7. Dilute 250 µL cell suspension with 5.75 mL Diluent.
8. Add 40 µL diluted cells to each well.
9. Place the lid on the plate, mix and incubate for 5 hours at 37 °C with 5% CO_2.

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

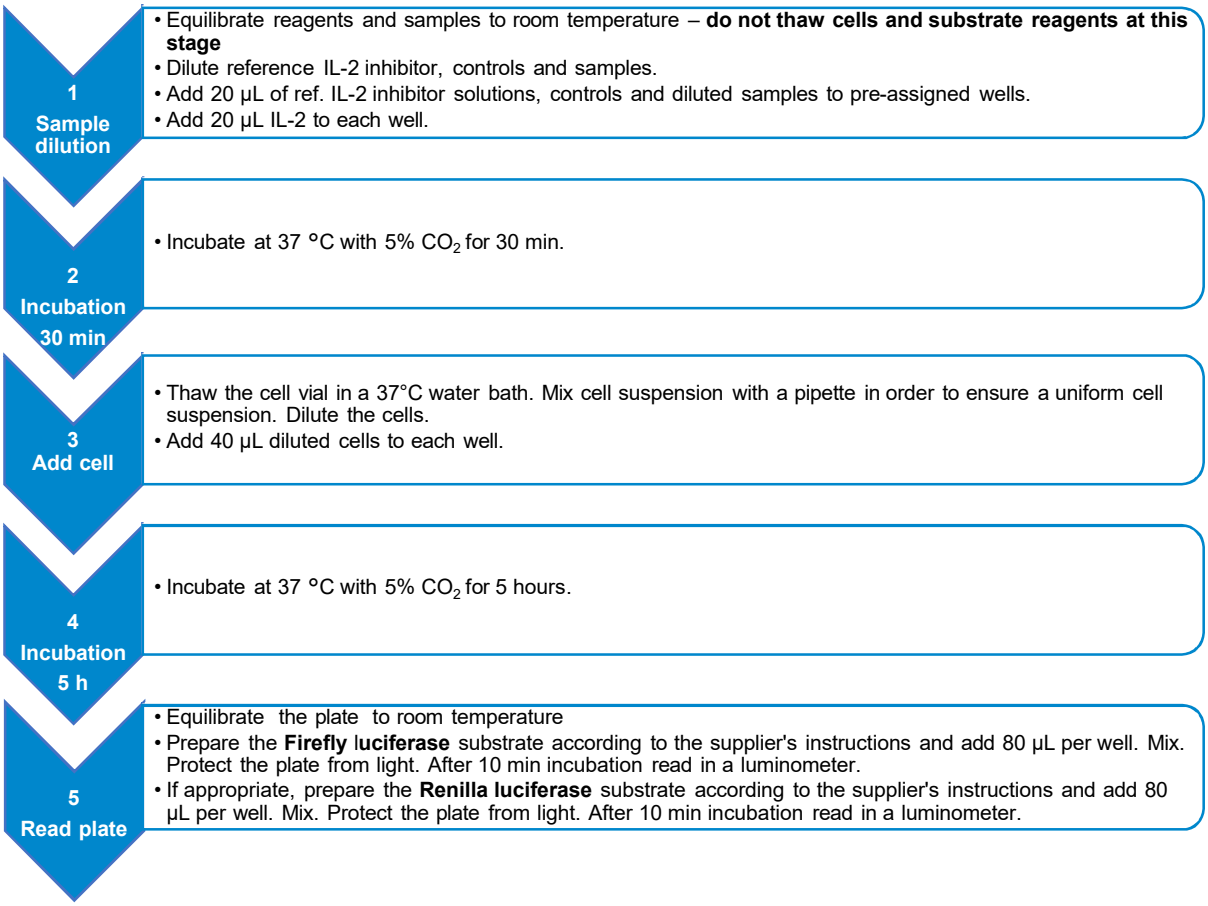
- This application note is intended for professional laboratory research use only. The data and results originating from following the Application Note should not be used either in diagnostic procedures or in human therapeutic applications.
- Use and handle the material and instruments referenced according 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-2 inhibitor activity using *iLite*® IL-2 Assay Ready Cells



Troubleshooting and FAQ

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

References

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2. Gaffen SL, Liu KD (November 2004). "Overview of interleukin-2 function, production and clinical applications". Cytokine. 28 (3): 109–23.