Quantification of type 1 Interferon inhibitor using *iLite*® Type I IFN 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

IFNα are widely used to treat chronic viral hepatitis in combination of anti-viral agents and in therapy of a wide variety of malignant diseases, including both some hematological malignancies and certain solid tumors. Many different preparations of IFNα are available commercially; the most commonly used formulations include IFNα2a and IFNα2b. (1,2) Several studies show correlation between development of anti IFNα neutralizing antibodies (NAbs) and loss of IFNα treatment efficacy. (3)

Interferon beta (IFNβ) is well established as a first line therapy in relapsing/remitting multiple sclerosis. (4,5) The occurrence of neutralizing antibodies (NAbs) and binding antibodies (BAbs) to IFNβ has been widely reported. Subjects with NAbs have shown reduced response to treatment with IFNβ, having higher relapse rates, increased MRI activity and higher risk of disease progression. (6)

Frequencies and titers of BAbs and NAbs vary depending on the preparation used, dose and frequency of administration and the assay used to quantify them. The *iLite*® platform offers a cell-based assay that enables the studies of Type I IFN (IFNα- and IFNβ-subtypes) interaction with their receptor and antibodies interfering with this interaction. (7)

Principle of the assay

The *iLite*® Type I IFN Assay Ready Cells are engineered cells optimized to engineered to express Firefly Luciferase under the control of an IFN α/β responsive promoter. When IFNα or IFNβ binds to the IFN α/β receptor on the cell surface, the IFN α/β regulated Firefly Luciferase reporter gene construct will be activated. The Firefly luciferase signal can be measured in a luminometer following addition and incubation of luciferase substrate. In the presence of inhibitory activity against IFN α/β, the amount of free IFN α/β is reduced, resulting in a decreased stimulation of Firefly luciferase production.

The Firefly luciferase signal is thus inversely proportional to the amount of inhibitory activity against IFNα or IFNβ in a sample. The *iLite*® Type I IFN Assay Ready Cells can therefore be utilized as an assay for quantification of Type I IFN inhibitor activity in test samples, including human serum.

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Material and equipment needed

Material and equipment	Suggested supplier	Reference
iLite® Type I IFN Assay Ready Cells	Svar Life Science	BM3049
Diluent (RPMI containing 9% heat inactivated FBS + 1% Penicillin-Streptomycin)	Gibco	61870-044 (RPMI) 26140-079 (FBS) 15140-122 (Penicillin-Streptomycin)
Rabbit anti-IFNβ (used in example protocol)	Peprotech	500-P32B
Type 1 IFN inhibitor	NA	NA
Human Interferon beta-1a (used in example protocol)	Prospec	CYT-236
Interferon α (subtype of choice)	NA	NA
Interferon β (subtype of choice)	NA	NA
Firefly substrate	Promega	E2620, Bright-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 type 1 IFN inhibitor (example given with anti-IFNβ, Peprotech 500-P32B)
Rabbit anti-IFNβ from Peprotech has successfully been used to neutralize IFNβ and inhibit the type 1 IFN regulated Firefly luciferase expression in *iLite*® Type I IFN Assay Ready Cells (refer to the table and graph below). **Note:** In this example protocol IFNβ-1a is used as stimulator and rabbit anti-IFNβ is used as inhibitor. At least 13 subtypes of IFNα and two subtypes of IFNβ is known to exist.

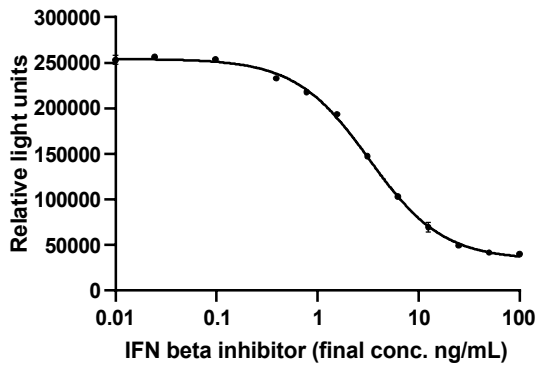


Figure 1. Example of IFNβ inhibitory curve

Final 20 IU/mL IFNβ-1a	Anti-IFNβ Suggested **solution conc.** ng/mL
A	300
B	150
C	75
D	38
E	19
F	9.4
G	4.7
H	2.3
I	1.2
J	0.29
K	0.073
L	0

Table 1. Suggested calibrator solution concentrations for anti-IFNβ 500-P32B

Incubation

1. Design a plate layout. It is recommended to perform the test at least in duplicate.
2. Perform a serial dilution of the reference type 1 IFN inhibitor. Ensure matrix consistency between reference antibody solutions, control solutions, and sample solutions.
3. Add 50 µL of the reference IFNβ inhibitor dilutions, controls and samples to assigned wells (final concentration will be one-third of solution concentration).
4. Add 50 µL of 60 IU/ml IFNβ to all wells (final concentration will be 20 IU/mL IFNβ).
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*® Type I IFN Assay Ready Cells in a 37°C water bath for 15 minutes. Invert the vial a minimum of 10 times to ensure a homogeneous distribution of cells.
7. Dilute 2 mL cells with 6 mL Diluent.
8. Add 50 µL diluted cells to each well.
9. Place the lid on the plate, mix and incubate for 18 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 50 µL per well. Mix and protect the plate from light. After 2 minutes incubation at room temperature read in a luminometer.

Precautions

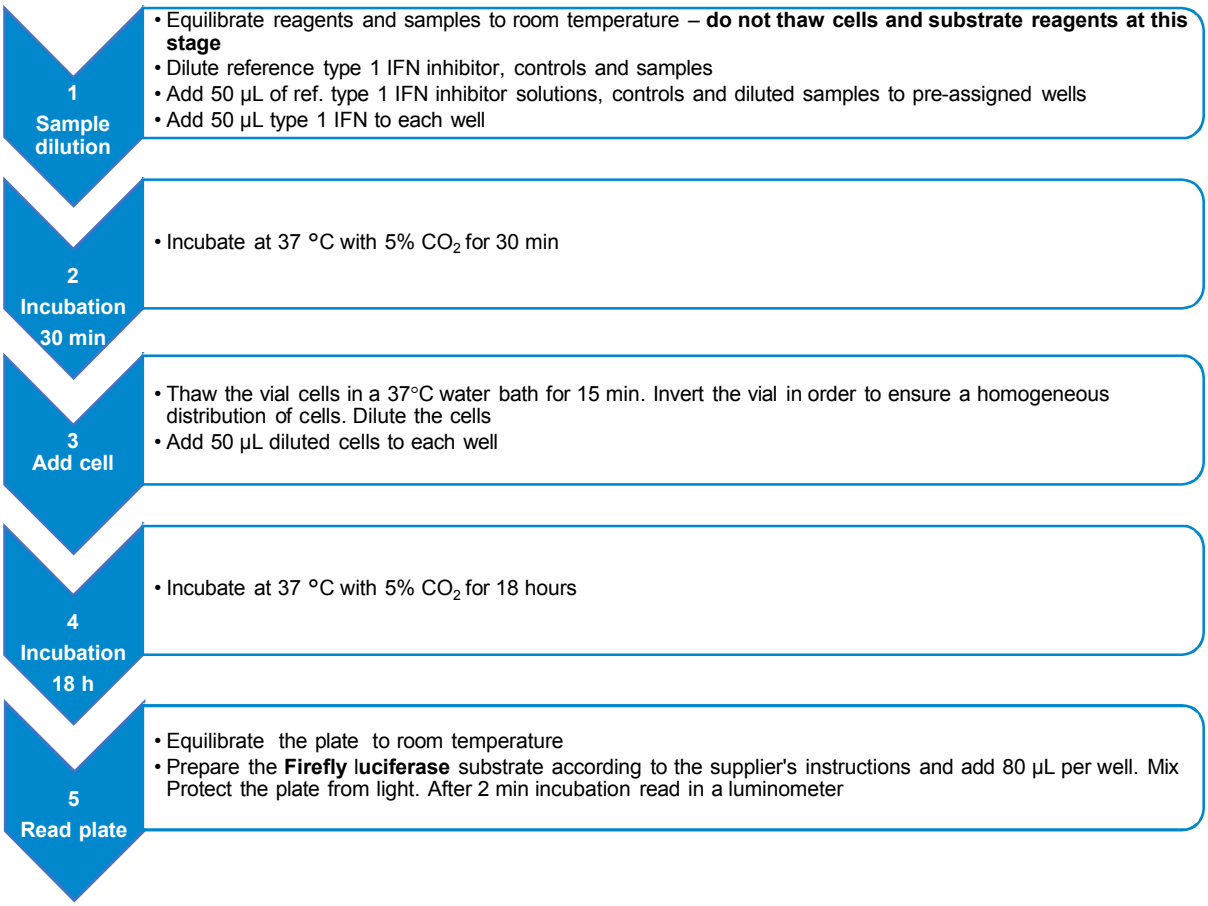
- This application note is intended for professional laboratory research use only. The data and results originating from following the Application Note should not be used either in diagnostic procedures or in human therapeutic applications.
- Use and handle the material and instruments referenced according 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 type 1 Interferon inhibitor activity using *iLite*® Type I IFN Assay Ready Cells



Troubleshooting and FAQ

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

References

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