Quantification of IFN beta inhibitor activity using *iLite*® IFNI FAST 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

Interferons (IFNs) are cytokines with antiviral, antitumor and immunoregulatory functions, released by both immune and non-immune cells as a first line of host innate defense, particularly viral infections. IFNs are classified into three groups and generally initiate signaling via the JAK-STAT (Janus kinase/signal transducer and activator of transcription) pathway. Type I IFNs includes at least 16 different subtypes, most well defined are IFN alpha (IFNα) and IFN beta (IFNβ) which binds to the heterodimeric IFN alpha/beta receptor (IFNAR) thereby inducing intracellular signaling (1,2). Type I IFNs are used in several clinical settings, due to their antiviral and immunomodulatory properties (3). These properties have generated interest in their clinical use to enhance antigen presentation, control viral infections and promote antitumor responses (3–5). Recently, a robust type I interferon response has been observed in patients with severe COVID-19, which could contribute to the exacerbated hyperinflammation observed in the progression to severe COVID-19 (6). Moreover, it has also been seen that vaccination together with IFN beta induces significantly greater expansion of tumor-specific CD8+ T cells than the other type I IFN subtypes tested (7).

Due to the detrimental effects of persistent viral infections and to improve biotechnological application (such as production of interferon-sensitive viruses for a range of applications), the development of IFN beta inhibitors has also been of interest (8). The iLite® IFNI FAST platform offers a reliable cell-based assay that enables studies of IFN beta inhibitors.

Principle of the assay

The *iLite*® IFNI FAST Assay Ready Cells are engineered cells, optimized to express Firefly luciferase (FL) under the control of a type I IFN responsive promoter. *iLite*® IFNI FAST 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 type I IFN 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 type I IFN in the sample, in this application note exemplified by IFN beta 1a. In the presence of inhibitory activity against IFN beta, the amount of free IFN beta 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 IFN beta in a sample.

Specimen collection

The *iLite*® IFNI FAST Assay Ready Cells can be used for quantification of IFN beta inhibitor activity in test samples, including human serum.

Material and equipment needed

Material and equipment	Suggested supplier	Reference
iLite® IFNI FAST Assay Ready Cells	Svar Life Science	BM4049
Diluent (DMEM containing 9% heat inactivated FBS + 1% Penicillin-Streptomycin)	Gibco	31966-021 (DMEM) 26140-079 (FBS) 15140-122 (Penicillin-Streptomycin)
Anti-human IFN beta antibody	Peprotech	500-P32B
Human IFN beta 1a (used in example protocol)	PBL Assay Scientific	11415-1
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 IFN beta inhibitor

Rabbit anti-human IFN beta antibody from Peprotech has successfully been used to neutralize IFN beta 1a and inhibit the IFN regulated Firefly luciferase expression in *iLite*® IFNI FAST Assay Ready Cells (refer to the table and graph below).

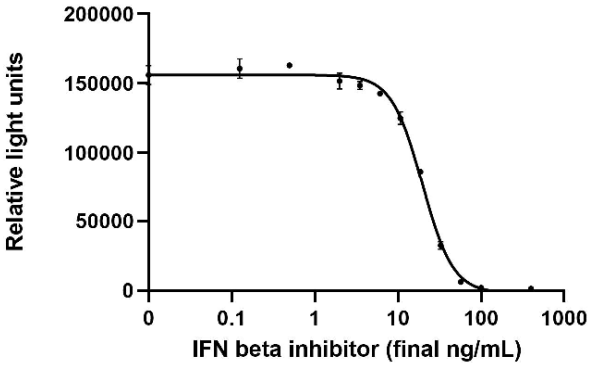

Figure 1. Example of IFN beta inhibitory curve

Final 70 IU/mL IFN beta 1a	Anti-IFN beta Ab Suggested calibrator solution concentrations, ng/mL
1	1600
2	400
3	229
4	131
5	75
6	43
7	24
8	14
9	8.0
10	2.0
11	0.50
12	0

Table 1. Suggested calibrator solution concentrations for IFN beta inhibitor

Assay preparation and 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 IFN beta inhibitor. Ensure matrix consistency between reference antibody solutions, control solutions, and sample solutions.
3. Add 20 µL of the reference IFN beta inhibitor dilutions, controls, and samples to assigned wells (final concentration will be a quarter of solution concentration).
4. Add 20 µL of 280 IU/ml IFN beta 1a to all wells (final concentration will be 70 IU/mL IFN beta 1a).
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*® IFNI FAST 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.
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 manufacturer'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.
12. If appropriate, prepare the **Renilla luciferase** substrate according to the manufacturer'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/ 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

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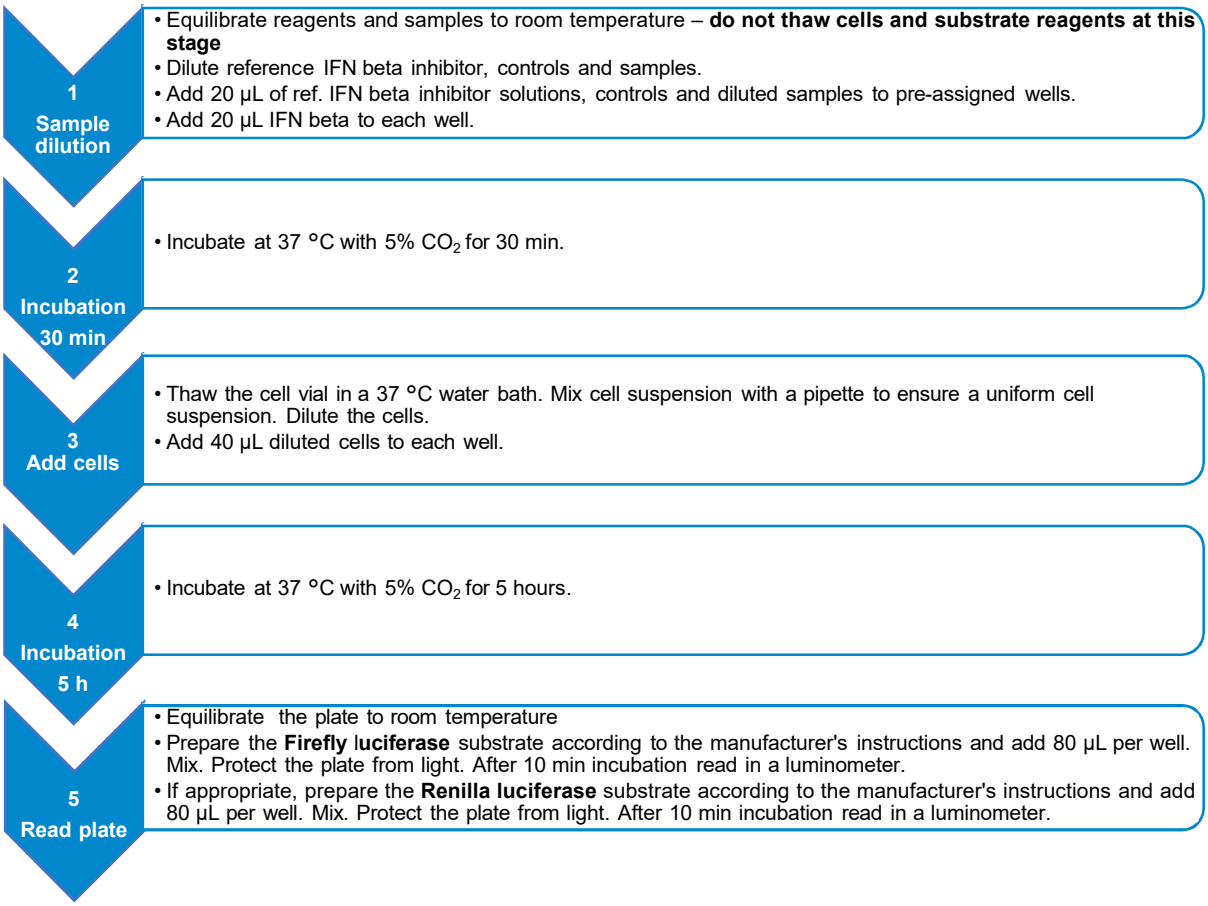

Troubleshooting and FAQ

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

References

1. Ivashkiv LB, Donlin LT. Regulation of type I interferon responses. Nat Rev Immunol. 2013;14(1):36–49.
2. Padilla CML de, Niewold TB. The type I interferons: Basic concepts and clinical relevance in immune-mediated inflammatory diseases. Gene. 2016;576(1):14–21.
3. Park A, Iwasaki A. Type I and Type III Interferons – Induction, Signaling, Evasion, and Application to Combat COVID-19. Cell Host Microbe. 2020;27(6):870–8.
4. Propper DJ, Balkwill FR. Harnessing cytokines and chemokines for cancer therapy. Nat Rev Clin Oncol. 2022;19(4):237–53.
5. Borden EC. Interferons α and β in cancer: therapeutic opportunities from new insights. Nat Rev Drug Discov. 2019;18(3):219–34.
6. Lee JS, Shin EC. The type I interferon response in COVID-19: implications for treatment. Nat Rev Immunol. 2020;20(10):585–6.
7. Audsley KM, Wagner T, Ta C, Newnes HV, Buzzai AC, Barnes SA, et al. IFNβ Is a Potent Adjuvant for Cancer Vaccination Strategies. Front Immunol. 2021;12:735133.

8. Gage ZO, Vasou A, Gray DW, Randall RE, Adamson CS. Identification of Novel Inhibitors of the Type I Interferon Induction Pathway Using Cell-Based High-Throughput Screening. J Biomol Screen. 2016;21(9):978–88.

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