Quantification of functional type I Interferon 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

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) and binds to the heterodimeric IFN alpha receptor (IFNAR) thereby inducing intracellular signaling. (1-3)

Type I IFNs are used in several clinically settings. 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. (4, 5) Several different preparations of IFNα are available commercially; the most commonly used formulations include IFNα-2a and IFNα-2b.
Interferon beta (IFNβ) was the first disease modifying therapy to be approved for the treatment of multiple sclerosis and is today well established as first line therapy. Both preparations of IFNβ-1a and IFNβ-1b are commercially available and used clinically. (6,7)

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. The Firefly luciferase signal is proportional to the concentration of functional IFNα or IFNβ in the sample (Fig.1).

Specimen collection

The *iLite*® Type I IFN Assay Ready Cells can be used for measuring concentration of IFNα or IFNβ in test samples including human serum.

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)
Human IFN 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% CO2	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 I IFN calibrator *(example protocol given with rec. human IFN β-1a, Prospec CYT-236)*

Interferon beta 1a from Prospec has successfully been used to stimulate the *iLite*® Type I IFN Assay Ready Cells. The below table shows the dilutions of IFNβ-1a, used for QC release of the *iLite*® Type I IFN Assay Ready Cells. **Note:** In this example protocol IFNβ-1a is used as stimulator. At least 13 subtypes of IFNα and two subtypes of IFNβ is known to exist. Stimulatory effect of BM3049 *iLite*® Type I IFN Assay Ready Cells has been shown with IFNα-2a (Pegasys), IFNα-2b (Pegintron), IFNβ-1a (Avonex and Rebif), and IFNβ-1b (Betaferon).

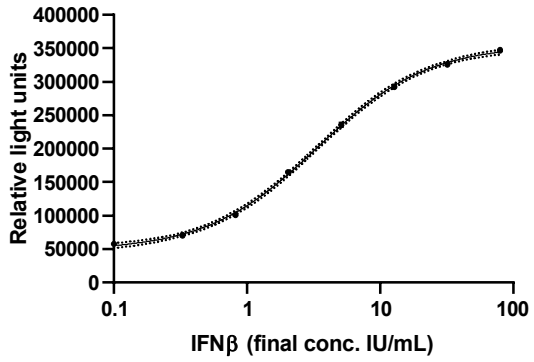

Figure 1. Example of IFNβ calibration curve.

Calibrator	IFNβ-1a Suggested **solution conc.** (IU/ml)
A	120
B	48
C	19
D	7.7
E	3.1
F	1.2
G	0.49
H	0

Table 1. Suggested calibrator **solution concentrations** for IFNβ-1a.

Incubation

1. Design a plate layout. It is recommended to perform the test at least in duplicate.
2. Dilute calibrators, controls and samples to fall within the expected **in assay values** (0-80 IU/mL in this example).
3. Add 100 µL calibrators, controls and samples in duplicate to assigned wells.
4. 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.
5. Dilute 2 mL cells with 6 mL Diluent.
6. Add 50 µL diluted cells to each well.
7. Place the lid on the plate, mix and incubate for 18 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 supplier's 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.

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

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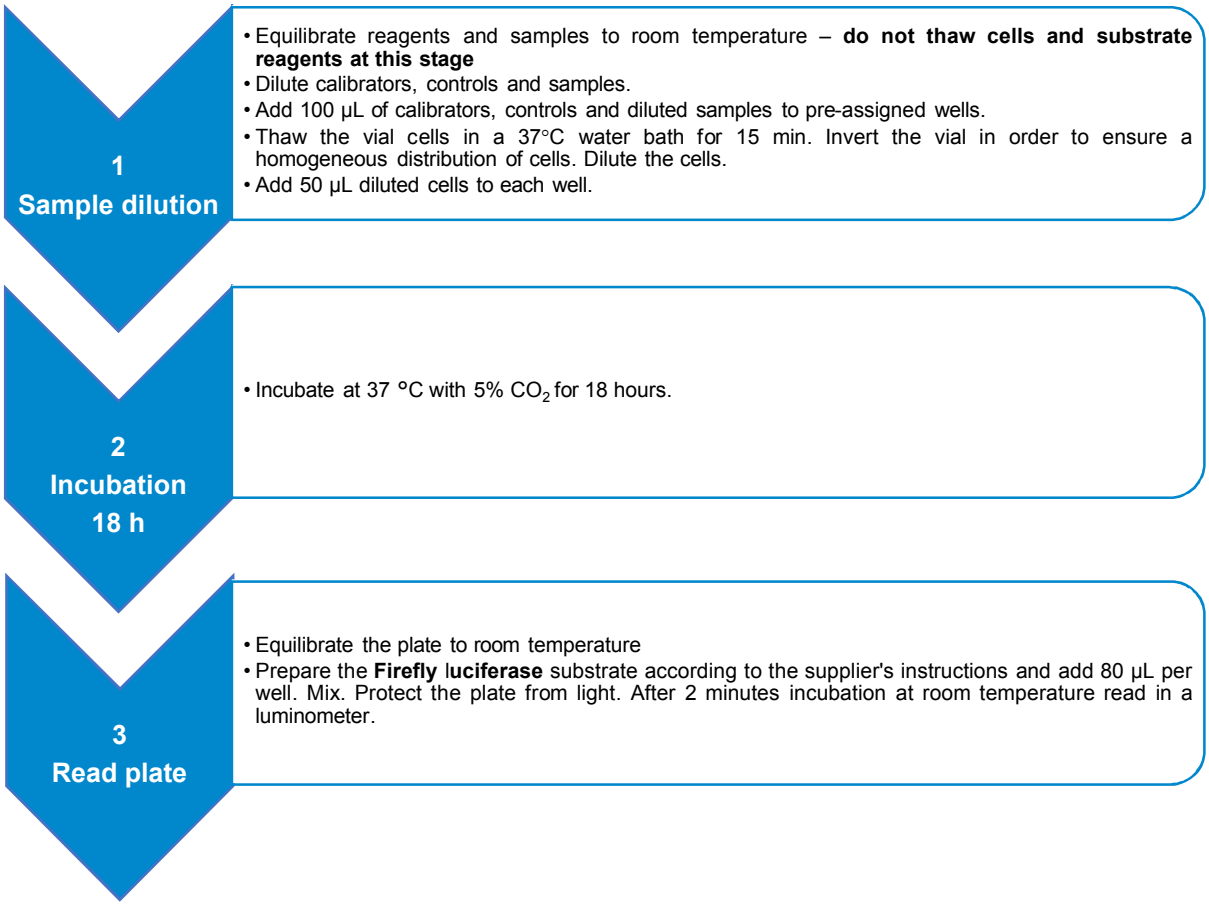

Troubleshooting and FAQ

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

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