# Quantification of Interferon α or β using *iLite™* Type I IFN Assay Ready Cells

*This application note contains a suggested protocol and performance data. Each individual laboratory must set up their own method and perform relevant validations.*
*For research and professional use only.*

## Background

Interferon alpha (IFNα) has been widely used to treat chronic viral hepatitis and a wide variety of malignant diseases, including hairy cell leukemia, basal cell carcinoma, chronic myeloid leukemia and cutaneous T-cell lymphoma. Several different recombinant preparations of IFNα are available commercially; the most commonly used formulations include IFNα2a and IFNα2b. A number of studies have shown that development of anti IFNα neutralizing antibodies (NAbs) is correlated with a loss of IFNα treatment efficacy.

Interferon beta (IFNβ) is well established as a first line therapy in relapsing/remitting multiple sclerosis. 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. The frequencies and titers of NAbs vary depending on the preparation used, dose and frequency of administration and also the assay used to quantify them.

The *iLite™* Type I IFN Assay Ready Cells can be used for measuring concentration of IFNα or β.

## Principle of the assay

The *iLite™* Interferon α/β Assay Ready Cells are engineered cells optimized to express Firefly luciferase under the control of an IFNα/β responsive promoter. When IFNα/β binds to the IFNα/β receptor on the cell surface it activates the IFNα/β regulated Firefly luciferase reporter gene construct. 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 IFNα/β in the sample (Fig.1).

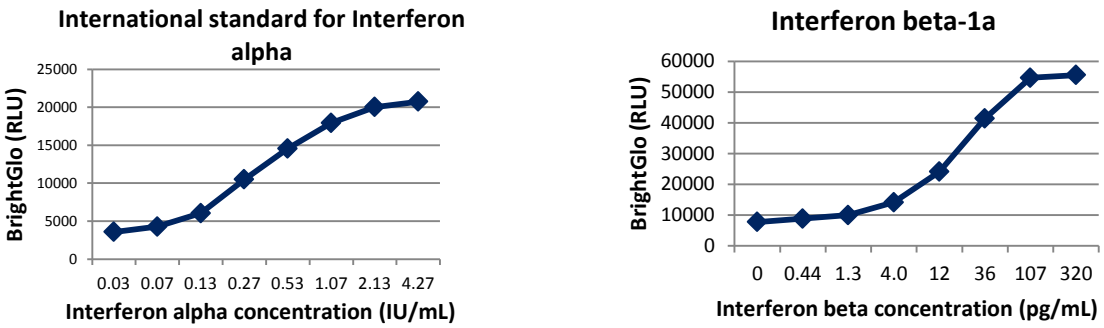



Fig 1. Concentration curves of Interferon alpha (left graph) and Interferon beta (right graph)

## Specimen collection

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

## Material and equipment needed

| Material and equipment | Suggested supplier | Reference |
|---|---|---|
| *iLite™ Type I IFN Assay Ready Cells* | Euro Diagnostica | BM3049 |
| Firefly luciferase substrate only | Promega | E2610, Bright-Glo™ Luciferase Assay System |
| Interferon α | NA | NA |
| Interferon β | NA | NA |
| Plate; White walled micro well plate suitable for luminescence | PerkinElmer | 6005680 |
| Microplate Luminometer with appropriate reading software – no filter on luminometer | Contact Euro Diagnostica 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 of cells | NA | NA |
| Single-use polypropylene reservoir | NA | NA |
| Plate shaker | NA | NA |
| Timer | NA | NA |

## Preparation of calibrators

Construct a calibrator curve by diluting Interferon to appropriate concentrations. A few examples can be found in Table 1. It is important to use a diluent that provides the same matrix content in all dilutions of calibrators and samples, i.e. the diluent might need to be complemented with serum. It is also important to establish an individual calibrator for every type of interferon used. A positive control should also be prepared from the Interferon used. Diluent is recommended as a negative control.

Table 1. Suggested calibrators concentrations for four Interferons

| Calibrator | Peginterferon alpha-2a | Peginterferon alpha-2b | International standard for Interferon alpha | Interferon beta-1a |
|---|---|---|---|---|
| | Calibrator conc. (IU/mL) | Calibrator conc. (IU/mL) | Calibrator conc. (IU/mL) | Calibrator conc. (pg/mL) |
| A | 0.2 | 0.033 | 0.033 | 0 |
| B | 0.4 | 0.07 | 0.07 | 0.44 |
| C | 0.7 | 0.13 | 0.13 | 1.3 |
| D | 0.9 | 0.27 | 0.27 | 4.0 |
| E | 1.2 | 0.53 | 0.53 | 12 |
| F | 1.6 | 1.1 | 1.1 | 36 |
| G | 2.1 | 2.1 | 2.1 | 107 |
| H | 2.7 | 4.3 | 4.3 | 320 |

## Protocol

### Sample dilution and pre-incubation

Measurement of Interferon α or β can be measured with a screening protocol (one dilution per sample and 40 samples per plate) or with a quantitation protocol (8 dilutions per sample and 5 samples per plate).

### Incubation

1. Design a plate layout.
2. Dilute all samples 1:2 for screening or in a dilution series for quantitation (recommended dilution factor is 2.5x in 8 dilution steps). It is important to use a diluent that provides the same matrix content in all dilutions, i.e. the diluent might need to be complemented with serum for dilution steps 2-8.
3. Add 100 µL calibrators and controls in duplicate to assigned wells
4. Add 100 µL samples to the assigned wells
5. Thaw the vial of cells in a 37°C water bath for 15 minutes. Invert the vial a minimum of 10 times to ensure a uniform cell suspension.
6. Dilute 2.5mL cells with 5.5mL diluent (ex. RPMI)
7. Add 50 µL diluted cells to each well.
8. Place the lid on the plate, mix and incubate for 18 hours at 37 °C with 5% $CO_2$.

### Adding substrate solutions

9. Thaw the vial of Bright-Glo™ Luciferase Assay System 30 minutes prior to completion of the incubation. Prepare the substrate according to the supplier's instructions and add 50 µL per well. Mix and protect the plate from light. Read in a luminometer after 2 minutes incubation at room temperature.

### Calculation of Interferon α or β of activity of unknown samples

Calculate the mean Relative Light Units (RLU) for each data point. Create a scatter plot for the calibrators with Interferon dilutions on the X-axis and linear RLU values on the Y-axis (Fig. 1). Apply a 4 parameter logistic curve fit and read the unknown samples against the curve. Each laboratory should establish its own positive/negative threshold and define the reproducibility.

### Quality Control

If the following criteria are met, the assay is considered valid:
Positive control: Positive for Interferon α/β
Negative control: Negative for Interferon α/β
% CV of duplicates ≤ 20%

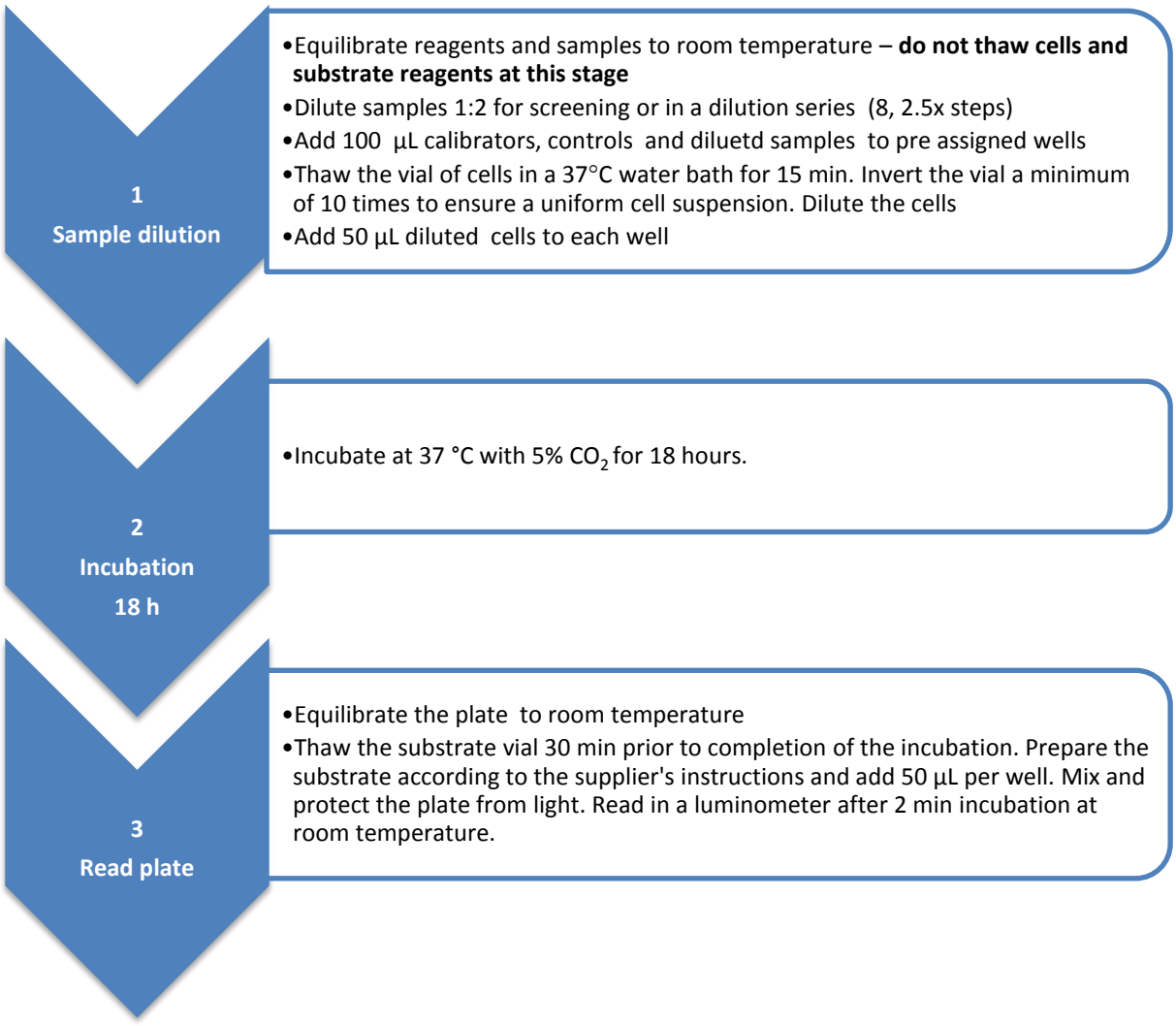
### Precautions

-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. Biomonitor *iLite™* cell-based products are covered by patents which are the property of Euro Diagnostica AB and any attempt to reproduce the delivered *iLite™* Assay Ready Cells is an infringement of these patents.

## Quick Guide – Quantification of Interferon α or β using *iLite™* Type I IFN Assay Ready Cells




## Troubleshooting and FAQ

Please consult Euro Diagnostica's website www.eurodiagnostica.com

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