Product #51100-1

***iLite*™ Human Interferon Alpha Kit**

Assay Range: 1.5 - 200 IU/ml (depending on the incubation period chosen, refer to step 7 of the assay procedure)

Incubation: 7 to 17 hours

Rev. 00

Intended Use: The Kit is intended for use as an *in vitro* test for the quantitative determination of Human Interferon Alpha (IFN-α) bioactivity (IU/ml) using luciferase generated-bioluminescence. Though this kit is provided with Human IFN Alpha 2b as the standard, the kit is also useful for examining the bioactivity of other interferons that bind to and activate the Type I receptor. These include the variety of IFN-α isoforms, IFN-β, IFN-κ and IFN-ω. This test is for research use only.

Background: Type I Interferons constitute a family of cytokines comprising at least 18 IFN-α isoforms, and single IFN-β, IFNκ and IFN-ω isotypes identified by their ability to protect cells against viral infection. They are produced by a wide variety of cells in response to a variety of stimuli, most notably in response to viral infection via the interaction of single-stranded or double-stranded viral RNA with Toll-like receptors (TLR) TLR8 and TLR3, respectively[1].

Assay Principle: The *iLite*™ Human IFN alpha assay is a quantitative Gene Reporter Bioassay. The test procedure is based on the sequential addition of diluent and sample to Human Type I interferon-sensitive cells in a bioluminescence microtiter plate. After incubation and addition of lysis reagent/substrate, the resultant bioluminescence intensity is proportional to the amount of Type I interferon activity (IU/ml) in the sample. The assay range is 1.5 IU/ml to 25 IU/ml or 6-200 IU/ml depending on the incubation period chosen.

Materials Provided:

A. *iLite*™ Cells
B. Interferon Standard (Hu IFN-α2b)
C. Assay Diluent
D. Steady-Glo® Luciferase Assay Buffer
E. Steady-Glo® Luciferase Assay Substrate (contains DTT)
F. 96-Well Sterile Microtiter Plate
G. Negative Control
H. Positive Control

Additional Materials Required (not provided):

- Micropipette (5-50µl)
- Multichannel Pipette (50-200µl)
- Polypropylene tubes (0.5-2.0ml)
- Microplate Luminometer
- Incubator 37°C, 5% CO_2
- Sterile Reservoir

Safety:

- *iLite*™ Human IFN alpha assay is for Research Use only.
- Wear protective clothing, disposable latex gloves and eye protection when performing the assay. Wash hands thoroughly when finished. If contact occurs rinse off immediately with water and seek medical advice.
- Residues of chemicals, kit components and specimens infected or potentially infected are generally considered as hazardous or biohazardous waste. All such materials should be disposed of in accordance with established safety procedures and good microbiological practice.
- The kit contains a stable transfected cell line derived from a commercially available pro-monocytic human cell line characterized by the expression of MHC Class II antigens, in particular, the human lymphocyte antigen (HLA-DR), on the cell surface. The cells are assigned Risk Group 1 (RG1). Work in a Class I or II biological safety cabinet.
- **CAUTION:** The cells contain less than 2.5% Dimethylsulfoxide (DMSO) and may be harmful if absorbed through skin, swallowed or inhaled. May cause irritation to skin, eyes and respiratory tract.
- **WARNING:** The Luciferase Assay Substrate contains dithiothreitol (DTT) and (as supplied) is classified as harmful if swallowed or inhaled. May be harmful if absorbed through skin. Causes irritation to skin, eyes and respiratory tract. May affect central nervous system.
- Visit our website at www.interferonsource.com for the Material Safety Data Sheet (MSDS).

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Receipt, Storage and Stability:

- Upon receipt, confirm that adequate dry ice is present and that the kit contents are frozen. Immediately transfer the kit to -80ºC storage
- All kit reagents are stored at -80ºC and are stable as supplied until the expiry date shown
- Cells should be used within 15 minutes of thawing
- Standard, Negative Control and Positive Control should be used within 30 minutes of thawing
- Assay Diluent should be used on the day of thawing
- Luciferase Assay Substrate should be used within 2 hours of reconstitution

Procedural Recommendations:

- The packaging integrity of the kit should be confirmed prior to use to confirm absence of reagent leaks
- To ensure kit performance, the protocol should be reviewed in its entirety prior to use
- Use the table in the assay procedure to design a plate layout prior to commencing the assay
- The Kit is for single use only
- Aseptic technique should be followed during assay set-up
- Do not use kit or individual reagents past their expiry date or substitute reagents from different kit lot numbers
- Deviation from the protocol provided may cause erroneous results
- Care must be taken not to contaminate components and always use fresh pipette tips for each sample and component
- All equipment should be calibrated prior to use
- Frozen components should be thawed per reagent preparation instructions (see below), and mixed appropriately prior to use to ensure homogeneity

Preparation of Reagents

Assay Diluent: Remove from -80°C storage and thaw at room temperature.

Micro Well Plate: Remove from -80°C and allow plate to equilibrate to room temperature.

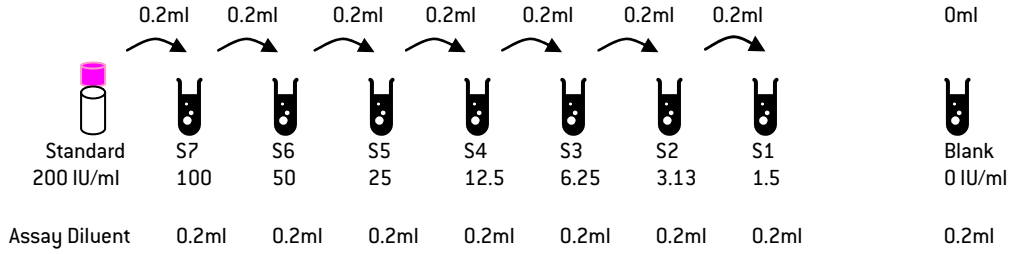
When the Assay Diluent has equilibrated, remove the following kit components from -80°C storage and allow thawing at room temperature:
IFN Standard, Negative Control, Positive Control

IFN Standard: Construct a standard curve from 0–100 IU/ml, or other appropriate range, by preparing a series of dilutions using the IFN Standard and Assay Diluent. Ensure adequate mixing of the thawed IFN Standard using a pipette.

a. Label eight polypropylene tubes (S1-S7 and blank). Alternatively, a microtiter plate can be used to prepare dilutions.
b. Fill tubes with Assay Diluent as indicated in the diagram that follows.
c. Using polypropylene tips, add the IFN Standard to S7 and mix gently.
d. Remove indicated amount from S7 and add to S6. Repeat series to S1. Change tips between each dilution.

Note: The IFN Standard, as supplied, may be used to generate a 200 IU/ml standard curve point if desired.

Standard Curve Serial Dilutions:



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Sample Preparation: Samples suspected to have an IFN activity greater than the linear range of the curve chosen should be appropriately diluted using the Assay Diluent. Samples should be diluted immediately prior to assay.

Luciferase Assay Lysis Buffer and Substrate: Refer to step 8 of the Assay Procedure.

Assay Procedure:

NOTE: All steps should be performed using aseptic technique

1. Rapidly thaw the vial of cells using a 37°C water bath. Thawing should be accomplished within 3-4 minutes. Gently invert vial a minimum of ten times to ensure a uniform cell suspension. Transfer cells to a sterile reservoir. **Do not vortex cells**.

2. Immediately transfer 25µl of the cell suspension to each well using a multichannel micro-pipette with sterile tips.

3. Add 50µl of Assay Diluent to each well. A multichannel micro-pipette may be used.

4. Place precisely 25µl of the Blank, IFN standard, Negative Control, Positive Control in individual wells of the microtiter plate (refer to Preparation of Reagents). Ensure adequate mixing of controls and of unknown samples using a pipette.

A recommended microtiter plate layout is presented below, with the IFN standards for the curve run in duplicate in columns 1 and 2. Additional replicates may be used if desired.

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	B	NC	NC								
B	S1	S1	PC	PC								
C	S2	S2										
D	S3	S3										
E	S4	S4										
F	S5	S5										
G	S6	S6										
H	S7	S7										
S= Standard B= Blank NC=Negative Control PC=Positive Control												

5. Add unknown samples (or controls) for testing, placing precisely 25µl into individual wells (columns 3 through 12), and recording the well position of each of the samples. It is recommended that unknowns be tested at least in duplicate.

6. Replace the lid on the micro well plate and incubate at 37°C in 5% CO_2.

7. Incubate the plate for the period required to achieve the desired sensitivity and linear range as follows:
Note: The standard preparation provides instruction for generation of a curve from 1.5-100 IU/ml. The IFN Standard, as supplied, may be used to generate a 200 IU/ml standard curve point if desired.

Incubation Period (hrs)	Detection Limit (IU/ml)	Linear Range (IU/ml)
7	6	6 to 200
12	1.5	1.5 to 200
17	0.8	1.5 to 25

See our website www.interferonsource.com for examples of calibration curves based on varying incubation times.

8. Within 1 hour of the desired incubation period, thaw the Luciferase Assay Lysis Buffer and Substrate by equilibrating at room temperature. Do NOT use a 37°C water bath. Once thawed, add 10 ml of Luciferase Lysis Buffer to the Substrate, replace the cap and mix gently by inversion.

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9. Add precisely 100µl of the reconstituted Luciferase Lysis Substrate per well using a multichannel pipette, taking care not to cross contaminate wells. Change pipette tips between additions of substrate to each row.

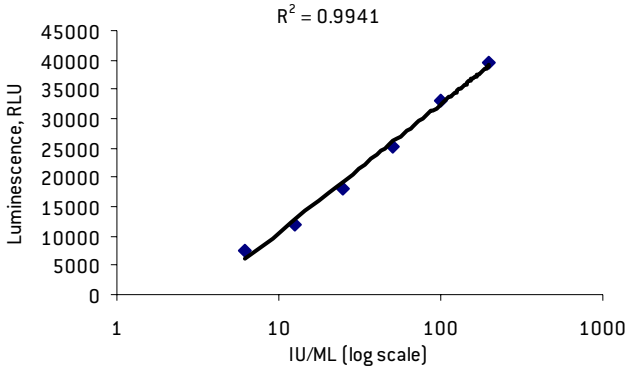
10. Read the plate on a Luminometer between 10 and 30 minutes after addition of reconstituted Luciferase Lysis Substrate to the plate. It is recommended that several readings of the plate be taken to ensure the lysis is complete and results have stabilized.

Calculation of Results:
Construct a standard curve by plotting the points within the detection limit of the incubation period chosen (refer to step 7).

- IFN Standard activity (IU/ml) on X axis (log scale)
- The mean of RLU for the IFN Standard (Human IFN Alpha 2b) on Y axis(linear scale)

Read the activity of interferon (IU/ml) indicated by the mean Relative Light Units (RLU) of the samples from the calibration curve.
For diluted samples, multiply the calculated interferon activity by the appropriate dilution factor to obtain the actual interferon activity.

Example Calibration Curve:
The following standard curve for Human Interferon Alpha 2b activity is provided as a demonstration only and should not be used to obtain test results. A standard curve must be run for each plate and set of samples assayed.



QC Criteria: If the criteria below are not met, the assay is considered invalid and must be repeated.

- The correlation coefficient for the trend line (R^2) of the standard curve must be ≥ 0.90
- Replicate RLU values for individual standard curve points must have a CV of ≤ 15%.

For Example:

Sample Activity	RLU	Mean RLU	CV
5 IU/ml	1600	1675	4.5%
	1750		

Limitations of Use: Samples containing material that could interfere with the bioluminescence determinations should be excluded (e.g., samples with a high lipid, bilirubin or hemoglobin content [visible by eye]).

References:

1. Iwasaki, A., & Medzhitov, R., Toll-like receptor control of the adaptive immune responses. *Nature Immunol.* 2004, 5, 987-995.

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