iLite™alphabeta
Human Type I Interferon Activity Detection Kit – Instructions For Use
The Biomonitor *iLite™alphabeta* kit is for Research Use Only.
This kit should NOT be used for diagnostic purposes.

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INTENDED USE:
The Kit is intended for use as an in-vitro test for the quantitative determination of Type I IFN levels using luciferase generated bioluminescence. 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-ß, IFNκ, IFN λ, and IFN-ω, isotypes identified by their ability to protect cells against viral infection. Type I Interferons are produced by a wide variety of cells 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™ alphabeta* 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 micro well plate. Post 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 IU/ml to 100 IU/ml.

Kit Components	Quantity
A: *iLite™* Cells	3.6 ml
B: *iLite™ Alphabeta* IFN stock solution (200 IU/ml)	400 µl
C: Diluent	13 ml
D: Assay Buffer	10 ml
E: Lysis Substrate (lyophilised) (contains dithiothreitol (DTT))	1 Vial
F: White-Walled Micro well Plate	1
G: Negative Control	200 µl
H: Positive Control	200 µl
I: Product Insert	1

PRECAUTIONS

SAFETY
- *iLite™ alphabeta* assay is for Research use only.
- *iLite™ alphabeta* assay is intended for use by qualified laboratory staff only.
- The kit contains a stable transfected cell line of human origin and all materials should be treated as potentially infectious.
- In accordance with EU regulations (90/219/EEC), the transfected cell line (*iLite™* cells) is classified as a Class 1 Genetically Modified microorganism (GMM), and should be handled and disposed of in a licensed contained-use facility in accordance with these regulations (biohazardous waste should be inactivated prior to disposal by autoclaving or using bleach). When used in accordance with the manufacturer's instructions the requirements of EC Directive 90/219/EEC of 23 April 1990 on the contained use of genetically modified micro organisms are deemed to have been met.
- Wear protective clothing, disposable latex gloves and eye protection when handling specimens and performing the assay. Wash hands thoroughly when finished. If contact occurs rinse off immediately with water and seek medical advice.
- Residues of chemicals, preparations and kit components are generally considered as biohazardous waste. All such materials should be disposed of in accordance with established safety procedures.
- The Luciferase Substrate contains dithiothreitol (DTT) and is therefore classified as hazardous. The reconstituted reagent is not known to present any hazard as the concentration of DTT is less than 1%. However, we recommend the use of laboratory protective clothing as described above when working with these reagents.
- Dispose of all specimens, infected or potentially infected material in accordance with good microbiological practice. All such materials should be handled and disposed as though potentially infectious.
- Do not pipette materials by mouth and never eat or drink at the laboratory workbench.

PROCEDURAL
- To ensure kit performance the protocol should be reviewed in its entirety prior to use.
- 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.
- Do not mix or substitute reagents from different kit lot numbers.
- Deviation from the protocol provided may cause erroneous results.
- Performing the assay outside the time and temperature ranges provided may produce invalid results. Assays not falling within the established time and temperature ranges must be repeated.
- 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.
- The packaging integrity of the kit should be confirmed prior to use to confirm absence of leaks.
- Before commencing the assay, an identification and distribution plan should be established.

RECEIPT, STORAGE AND STABILITY
- Upon receipt confirm that adequate dry-ice is present and the kit is frozen. Immediately transfer to **minus 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.
- Standards should be used within 30 minutes of thawing.
- Diluent should be used on the day of thawing.
- Luciferase reagent should be used within 2 hours of reconstitution (or within 2 weeks if frozen at -20ºC)

ADDITIONAL MATERIALS REQUIRED
- Micropipette (5µl-50µl) and a multichannel pipette (50µl-200µl).
- Micro-plate luminometer (e.g. Victor ™ Light luminometer, PerkinElmer LAS, Seer Green, Bucks, UK).
- Liquid trough.
- Incubator 37ºC, 5% CO_2.

PREPARATION OF REAGENTS
After the incubation step has been completed, thaw the assay buffer and substrate at room temperature (Do NOT use a 37°C water bath). Add 10 ml of SteadyGlo assay buffer to the substrate, replace cap and mix gently by inversion. The preparation is now ready for addition to the micro well plate.

SAMPLE PREPARATION
Samples suspected to have an IFN activity greater than 100 IU/ml, should be appropriately diluted using the kit Diluent. Samples should be diluted immediately prior to assay.

ASSAY PROCEDURE
1. Remove diluent (C) and thaw at room temperature. The micro well plate (F) should also be allowed to equilibrate at room temperature at this time.
2. When the diluent has equilibrated, the following kit components are removed from – 80ºC storage and allow to thaw at room temperature:
    - IFN Stock Solution (kit component B),
    - Negative Control (G)
    - Positive Control (H)

3. Construct a standard curve from 0–100 IU/ml by preparing a series of dilutions using the IFN stock solution (B) and Diluent (C). Ensure adequate mixing of thawed stock using a pipette. Label 8 polypropylene tubes and serially dilute the IFN stock (B) as directed in table 2. Pipette tips should be changed between each dilution. Alternatively the user may prepare the dilutions using a micro well plate (not provided).

Tube or Well No.	S1	S2	S3	S4	S5	S6	S7	S8
Volume **(µl)**	200 Stock (B)	200 S1	200 S2	200 S3	200 S4	200 S5	200 S6	0
Diluent **(µl)**	200	200	200	200	200	200	200	200
Final Activity **(IU/ml)**	100 IU/ml	50 IU/ml	25 IU/ml	12.5 IU/ml	6.25 IU/ml	3.13 IU/ml	1.5 IU/ml	0 IU/ml

Table 2: IFN stock dilution scheme recommended for construction of standard curve.

4. Rapidly thaw the vial of cells using a 37°C water bath. Thawing should be accomplished within 3-4 minutes. Invert vial minimum of 10 times to ensure a uniform cell suspension, transfer cells to a sterile multichannel trough.

Note: Steps 5-9 should be conducted following aseptic technique.

5. Immediately transfer 25µl of the cell suspension to each well using a multichannel micro-pipette with sterile tips.
6. Add 50 µl of Diluent to each well (a multichannel micro-pipette may be used).
7. Samples of unknown IFN activity to be tested should be readied at this point, and diluted using Diluent (C) if required.
8. Place precisely 25µl of the IFN standards (prepared in step 2), Negative Control, Positive Control or Unknown samples in individual wells of the micro titre plate (columns 3 through 12), recording the well position of each of the samples. It is recommended that unknowns be tested in at least duplicate. Ensure adequate mixing of controls and unknown samples using a pipette.

A recommended micro 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	S8	S8	NC1	NC1								
B	S7	S7	PC1	PC1								
C	S6	S6										
D	S5	S5										
E	S4	S4										
F	S3	S3										
G	S2	S2										
H	S1	S1										

S= Standard, NC=Negative Control, PC=Positive Control

9. Replace the lid on the micro well plate and incubate at 37°C in 5% CO_2. for the time period selected.

The following incubation periods are recommended depending on user's desired sensitivity and linear range

- 7 hour incubation: Detection limit of 6.25 IU/ml, Linear Range: 6.25 to 200 IU/ml
- 17 hour incubation: Detection limit of 1.6 IU/ml, Linear Range: 1.6 to 25 IU/ml

See our website www.biomon.dk for examples of calibration curves based on varying incubation times.

10. At the end of the incubation period thaw the assay buffer (D) and substrate (E) at room temperature. Note: Do **NOT** use a 37°C water bath.
11. Add 10 ml of assay buffer (D) to substrate (E), replace cap and mix gently by inversion.
12. Add precisely 100µl of the reconstituted Lysis substrate per well using a multichannel pipette, taking care not to cross contaminate wells.
13. Change pipette tips between additions of substrate to each row
14. Read the plate on a luminometer, between 5 (min) and 30 (max) minutes after addition of reconstituted lysis substrate to the plate.

CALCULATIONS OF RESULTS

Construct a standard curve by plotting:

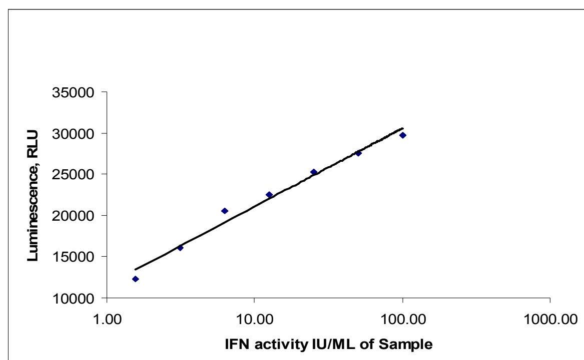
i. IFN activity on X –axis (log scale)
ii. The mean of RLU for each of the IFN Standards for Hu IFN-α on Y scale (linear scale).

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 OF CALIBRATION CURVE

The following standard curve for Human Type I IFN 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%.

e.g.

Sample Activity	RLU	Mean RLU	CV
5 IU/ml	1600	1675	6.3%
	1750		

- Mean RLU for lowest Standards must be greater than mean RLU negative (0 IU/ml standard) +2sd.

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 haemoglobin content (visible by eye)).

WARRANTY

The performance data presented here was obtained using the procedure described. Any change or modification of the procedure, not recommended by Biomonitor Ltd, may affect the results, in which case Biomonitor Ltd disclaims all warranties, expressed, implied or statutory, including implied merchantability and fitness for use. In the case of such an event, Biomonitor Ltd shall not be liable for damages, direct or consequential.

References:

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