# IFN-α/β Detection Assay Using Sensor Cell Lines

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**[Abstract]** Type I interferons (IFN-α/β) play an important role in host resistance to viral infections. Signaling through the JAK-STAT pathway, IFN-α/β stimulates response elements (ISRE) in the promoters of ISG to regulate their expression [reviewed in Randall *et al.* (2008)]. This method was adapted from InvivoGen to specifically detect and quantify IFN-α/β secreted in response to virus infection. HEK-Blue™ IFN-α/β cells were generated by stably introducing the human STAT2 and IRF9 genes into HEK293 cells to obtain a fully active type I IFN signaling pathway. The activation of this pathway is made detectable by the addition of a reporter gene expressing a secreted embryonic alkaline phosphatase (SEAP) under the control of the ISG54 promoter. ISG54 is a well-known ISG activated through an ISRE-dependent mechanism by type I IFNs.

## Materials and Reagents

1. HEK-Blue™ IFN-α/β cells (InvivoGen, catalog number: hkb-ifnab)
2. QUANTI-Blue (InvivoGen, catalog number: rep-qb2)
3. IFN-α2 (PBL Biomedical Laboratories, catalog number: PBL 11105-1)
4. Dulbecco's Modified Eagle's Medium (DMEM) (PAA Laboratories GmbH)
5. Fetal Calf Serum (FCS) (Biochrom)
6. PBS (Naxo OÜ)
7. Trypsin/EDTA (GE Healthcare)
8. 100x Penicillin-Streptomycin (Naxo OÜ)
9. Normocin (InvivoGen, catalog number: ant-nr-1)
10. Zeocin (InvivoGen, catalog number: ant-zn-1)
11. Blasticidin (Sigma-Aldrich, catalog number: 3513-03-9)

## Equipment

1. 96-well plate
2. 37 °C, 5% $CO_2$ cell culture incubator
3. Microscope
4. UV cross-linker (Hoefer, model: UVC5000)

5. Tecan Sunrise™ microplate reader

**Procedure**

A. Cells and media

HEK-Blue™ IFN-α/β cells were maintained in DMEM containing 10% heat-inactivated FCS, 50 U/ml penicillin, 50 µg/ml streptomycin, and 100 µg/ml Normocin in a humidified incubator at 37 °C under 5% $CO_2$. HEK-Blue™ IFN-α/β cells should not be passaged more than 20 times to remain fully efficient. HEK-Blue™ IFN-α/β cells should be maintained with two selective antibiotics, Zeocin (100 mg/ml) and Blasticidin (30 µg/ml). Do not use selective antibiotics for the test procedure. Cells should be passaged when a 70-80% confluency is reached.

B. Interferon detection

Day 1

1. Add 50 µl of each sample per well of a flat-bottom 96-well plate. If infectious virus is present in the collected samples, it should be inactivated using UV cross-linker for 5 min at 1,000 µJ/$cm^2$.

   The sample of the 96-well plate with standards, samples and negative controls (see next steps for the details). “Mock” indicates the same amount of media, but from uninfected cells. “Neg.c.” indicates negative control and described below.

| IFN (1) | IFN (1) | IFN (1) | Sample 1 | Sample 1 | Sample 1 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| IFN (2) | IFN (2) | IFN (2) | Sample 2 | Sample 2 | Sample 2 | | | | | | |
| IFN (3) | IFN (3) | IFN (3) | Sample 3 | Sample 3 | Sample 3 | | | | | | |
| IFN (4) | IFN (4) | IFN (4) | Sample 4 | Sample 4 | Sample 4 | | | | | | |
| IFN (5) | IFN (5) | IFN (5) | Sample 5 | Sample 5 | Sample 5 | | | | | | |
| IFN (6) | IFN (6) | IFN (6) | Mock | Mock | Mock | | | | | | |
| IFN (7) | IFN (7) | IFN (7) | Neg.c. 1 | Neg.c. 1 | Neg.c. 1 | | | | | | |
| IFN (8) | IFN (8) | IFN (8) | Neg.c. 2 | Neg.c. 2 | Neg.c. 2 | | | | | | |

2. Add 50 µl of IFN-α or IFN-β dilutions (1-8).

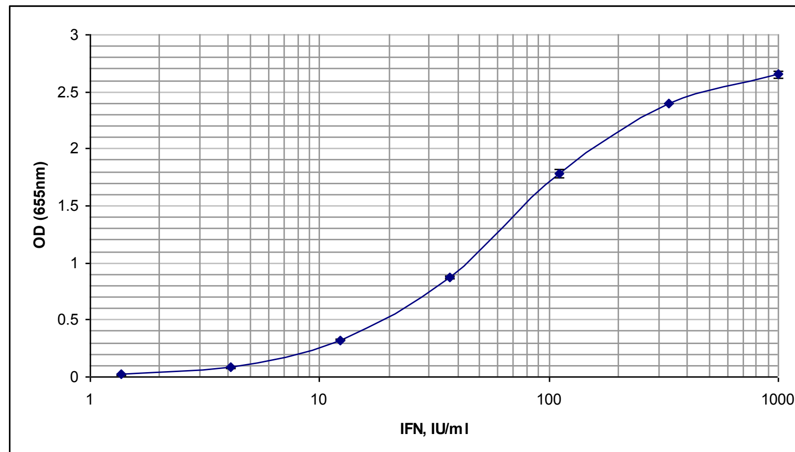
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| 10,000 U/ml | 3,333 U/ml | 1,111 U/ml | 370 U/ml | 123 U/ml | 41 U/ml | 13.7 U/ml | 4.6 U/ml |
| 75 µl of $10^4$ IU/ml | 50 µl DMEM | 50 µl DMEM | 50 µl DMEM | 50 µl DMEM | 50 µl DMEM | 50 µl DMEM | 50 µl DMEM |

Transfer 25 µl to each next well to make 1:3 dilutions as demonstrated in the table above. To prepare the dilutions, use the same DMEM as for the maintenance of HEK-Blue™ IFN-α/β cells (no selective antibiotics needed for this step).

3. Suggested negative controls: (1) 50 µl of IFN-α/ at $10^4$ U/ml in one well, (2) 50 µl of UV-inactivated medium from virus infected IFN-α/β-negative cell line (like BHK-21) with titer of virus similar to samples used in the same experiment.
4. Prepare suspension of HEK-Blue™ IFN-α/β cells at ~280,000 cells per ml in test medium (DMEM containing 10% heat-inactivated FCS, 50 U/ml penicillin, 50 µg/ml streptomycin, and 100 µg/ml Normocin).
5. Add 180 µl of cell suspension (~50,000 cells) per well.
6. Incubate the plate at 37 °C in a $CO_2$ incubator for 20-24 h.

Day 2

1. Prepare QUANTI-Blue™ reagent following the instructions on the package.
2. Add 180 µl of resuspended QUANTI-Blue™ reagent per well of a flat-bottom 96-well plate.
3. Add 50 µl of supernatant from induced HEK-Blue™ IFN-α/β cells.
4. Incubate the plate for 1-3 h at 37 °C in $CO_2$ incubator.
5. Determine SEAP levels using a spectrophotometer (Tecan Sunrise plate reader) at 620-655 nm.
6. Calculations should be performed using plots drawn separately for each range of obtained values. Please note that the curve is not linear and IFN values should be within the detection range, otherwise use dilutions of initial samples.
   Representative curve obtained in one of the experiments is shown below. Please note that in given experimental conditions higher concentrations of IFN (1,000-10,000) reach the plateau level and dilutions of initial samples are strongly recommended:




*Note: manufacturer recommends using 20 µl of sample volume (day 1) and 20 µl of supernatant (day 2), but we found that increased volumes (50 µl) improved the reproducibility of the results.*

## Notes

1. All specific product information and guidelines can be found at the manufacturer's website:
   http://www.invivogen.com/hek-blue-ifn-ab.
2. Cell line stability
   It is critical to prepare an adequate number of frozen stocks at early passages. HEK-Blue™ IFN--α/β cells should not be passaged more than 20 times to remain fully efficient. HEK-Blue™ IFN--α/β cells should be maintained in growth medium supplemented with the two selective antibiotics, Zeocin™ (100 g/ml) and Blasticidin (30 µg/ml). Antibiotic pressure with Blasticidin is required to maintain the plasmid coding for human STAT2 and IRF9 genes, and Zeocin™ is required to maintain the plasmid coding for SEAP.

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**References**

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