## Validation data for HEK-Blue<sup>TM</sup> IFN- $\alpha/\beta$ cells

https://www.invivogen.com/hek-blue-ifn-ab

## For research use only

Version 23I12-AK

HEK-Blue<sup>TM</sup> IFN- $\alpha/\beta$  cells allow the detection of bioactive type I interferons IFN- $\alpha$  and IFN- $\beta$  by monitoring the activation of the JAK/STAT/ ISGF3 pathway. These cells were generated by the stable transfection of HEK293 cells with the human STAT2 and IRF9 genes to obtain a fully active IFN- $\alpha/\beta$  signaling pathway. They also express a SEAP reporter gene under the control of the IFN-inducible ISG54 promoter. These cells respond strongly to both human IFN- $\alpha$  and IFN- $\beta$  (Figure 1). Of note, HEK-Blue<sup>TM</sup> IFN- $\alpha/\beta$  cells do not respond to either type III IFNs (IFN- $\lambda$ ) or type II IFN (IFN- $\gamma$ ). However, they respond poorly to murine (m) type I IFNs (Figure 2). These cells can also be used to screen for molecules that inhibit IFN- $\alpha/\beta$  signaling, such as antibodies targeting IFN- $\alpha$  (Figure 3).

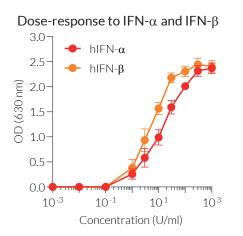


Figure 1. Dose-response of HEK-Blue<sup>TM</sup> IFN- $\alpha/\beta$  cells to recombinant type I IFNs. Cells were stimulated with increasing concentrations of recombinant human (h)IFN- $\alpha$  and hIFN- $\beta$ . After overnight incubation, the ISGF3 response was determined using QUANTI-Blue<sup>TM</sup> Solution, a SEAP detection reagent. The optical density (OD) at 630 nm is shown as mean ± SEM.

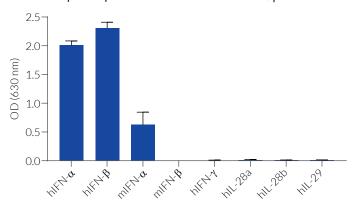
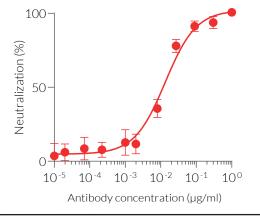


Figure 2. Response of HEK-Blue<sup>m</sup> IFN- $\alpha/\beta$  cells to a panel of cytokines. Cells were stimulated with various human recombinant cytokines: 100 U/ml hIFN- $\alpha$ 2b or hIFN- $\beta$ -1a, 10000 U/ml mIFN- $\alpha$  or mIFN- $\beta$ , 100 ng/ml IFN- $\gamma$ , 100 ng/ml IL-28a, IL-28b, or IL-29. After overnight incubation, SEAP activity was assessed using QUANTI-Blue<sup>m</sup> Solution. The optical density (OD) at 630 nm is shown as mean ± SEM.



## Neutralization of IFN- $\alpha$ response using anti-hIFN- $\alpha$ mAb

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Figure 3. Dose-dependent inhibition of HEK-Blue<sup>M</sup> IFN- $\alpha/\beta$  cell response using Anti-IFN- $\alpha$ -IgG. A serial dilution of Anti-IFN- $\alpha$ -IgG monoclonal antibody (mAb) was incubated with 500 U/ml of recombinant human IFN- $\alpha$ 2b for 30 minutes prior to the addition of the HEK-Blue<sup>M</sup> IFN- $\alpha/\beta$  cells. After overnight incubation, the ISGF3 response was determined using QUANTI-Blue<sup>M</sup> Solution, a SEAP detection reagent. Data are presented as percentage of neutralization (mean ± SEM).



## Response profile of HEK-Blue<sup>M</sup> IFN- $\alpha/\beta$ cells