Validation data for HEK-BlueTM IFN- α/β cells

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Version 23I12-AK

HEK-BlueTM IFN- α/β cells allow the detection of bioactive type I interferons IFN- α and IFN- β 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- α/β 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- α and IFN- β (Figure 1). Of note, HEK-BlueTM IFN- α/β cells do not respond to either type III IFNs (IFN- λ) or type II IFN (IFN- γ). 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- α/β signaling, such as antibodies targeting IFN- α (Figure 3).

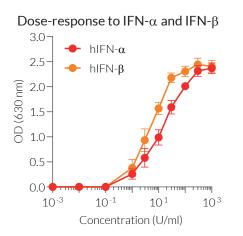


Figure 1. Dose-response of HEK-BlueTM IFN- α/β cells to recombinant type I IFNs. Cells were stimulated with increasing concentrations of recombinant human (h)IFN- α and hIFN- β . After overnight incubation, the ISGF3 response was determined using QUANTI-BlueTM Solution, a SEAP detection reagent. The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

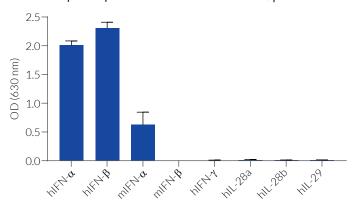
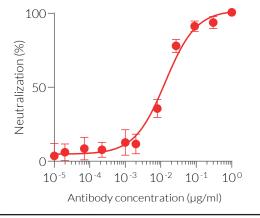


Figure 2. Response of HEK-Blue^m IFN- α/β cells to a panel of cytokines. Cells were stimulated with various human recombinant cytokines: 100 U/ml hIFN- α 2b or hIFN- β -1a, 10000 U/ml mIFN- α or mIFN- β , 100 ng/ml IFN- γ , 100 ng/ml IL-28a, IL-28b, or IL-29. After overnight incubation, SEAP activity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



Neutralization of IFN- α response using anti-hIFN- α mAb

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



Response profile of HEK-Blue^M IFN- α/β cells