Validation data for B16-Blue™ IFN-α/β cells

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Version 21F18-MM

B16-Blue™ IFN-α/β cells allow the detection of bioactive murine type I interferons (i.e. mIFN-α and mIFN-β) by monitoring the activation of the JAK/STAT/ISGF3 pathway and/or IRF3 pathway. These cells derive from the murine B16 melanoma cell line of C57BL/6 origin after stable transfection with a SEAP (secreted embryonic alkaline phosphatase) reporter gene under the control of the IFN-α/β-inducible ISG54 promoter. B16-Blue™ IFN-α/β cells respond specifically to mIFN-α/β in a dose-dependent manner (figure 1) and do not respond to human IFN-α/β (figure 2). Furthermore, due to the inactivation of the IFN-γ receptor, they do not respond to mIFN-γ (figure 2). Stimulation of these cells with type I IFN inducers, such as poly(dA:dT) delivered intracellularly, triggers the production of SEAP by the activation of the IRF-inducible promoter (figure 2).

Figure 1. Dose-response of B16-Blue™ IFN-α/β cells to recombinant murine IFN-α/β. Cells were stimulated with increasing concentrations of recombinant murine IFN-α/β (also known as mIFN-α3 and mIFN-β). After overnight incubation, the ISGF3 response was determined using QUANTI-Blue™ Solution, a SEAP detection reagent, and reading the optical density (OD) at 630 nm. The OD at 630 nm is shown as mean ± SEM.

Figure 2. Response of B16-Blue™ IFN-α/β cells to a panel of cytokines. Cells were stimulated with various human and murine recombinant cytokines: 1000 IU/ml mIFN-αA, mIFN-β, human IFN-α2a (hIFN-α2a), hIFN-β, 100 ng/ml of mIFN-λ, mIFN-γ, hIFN-γ, or 1 µg/ml of the type I IFN inducer poly(dA:dT) complexed extemporaneously with the transfection reagent LyoVec™ (Poly(dA:dT)/LV). After overnight incubation, SEAP activity was assessed using QUANTI-Blue™ Solution. The OD at 630 nm is shown as mean ± SEM.