Validation data for HEK-Blue™ IL-33 cells

https://www.invivogen.com/hek-blue-il33

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Version 21F28-MM

HEK-Blue™ IL-33 cells allow the detection of bioactive human interleukin-33 (hIL-33) by monitoring NF-κB and AP-1 activation. These cells were generated by stable transfection of human embryonic kidney 293 (HEK293)-derived cells with the human IL1RL1 gene (a subunit of the heterodimeric IL-33 receptor; the other subunit IL-1RAcP is naturally expressed in these cells). They were also stably transfected with an NF-κB/AP-1-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. In addition, the IL-1β and hTNF-α responses have been blocked, hence, enabling HEK-Blue™ IL-33 cells to respond specifically to IL-33.
HEK-Blue™ IL-33 cells display high sensitivity to hIL-33 (detection range: 0.3 - 100 ng/ml; **figure 1**). They are non-responsive to hIL-1β, murine IL-1β (mIL-1β), hIL-2, hIL-4, hIL-17, hIL-18, hIFN-α, hIFN-β, hIFN-γ, and hTNF-α (**figure 2**). Of note, while the response to hTNF-α has been blocked, the response to mTNF-α remains intact (**figure 2**). Furthermore, HEK-Blue™ IL-33 cells can be used for screening antibodies targeting the IL-33 pathway (**figure 3**).

Cellular response to IL-33

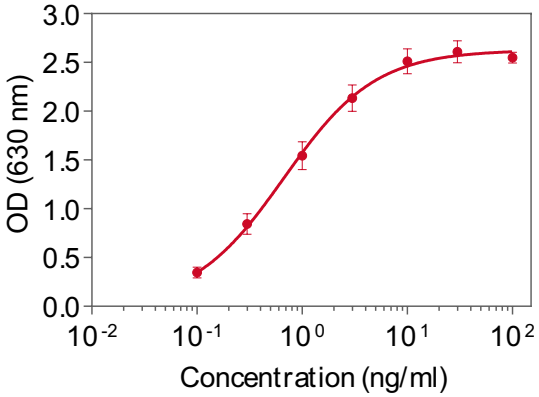

Figure 1. Dose-response of HEK-Blue™ IL-33 cells to recombinant hIL-33. Cells were stimulated with increasing concentrations of recombinant IL-33. After overnight incubation, the NF-κB/AP-1 response was determined using QUANTI-Blue™ Solution, a SEAP detection reagent, and reading the optical density (OD) at 630 nm. Data are shown as mean ± SEM.

Human IL-33 signaling inhibition

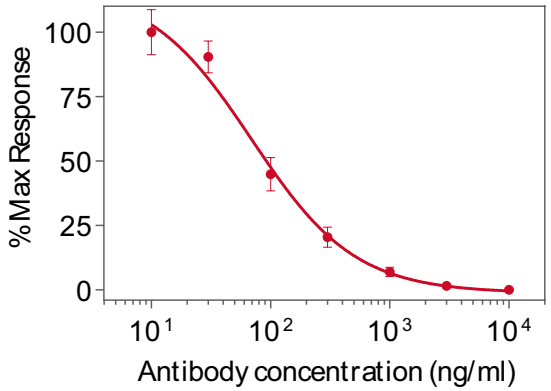

Figure 3. Dose-dependent inhibition of HEK-Blue™ IL-33 cellular response using a neutralizing antibody against hIL-33. The anti-hIL33 antibody was incubated with the cells for 30 minutes prior to the addition of hIL-33 (1 ng/ml). After overnight incubation, SEAP activity in the cell culture supernatant was assessed using QUANTI-Blue™ Solution. Data (shown as mean ± SEM) represent percentage (%) of maximal reporter activity without the anti-hIL33 antibody.

Cell line specificity

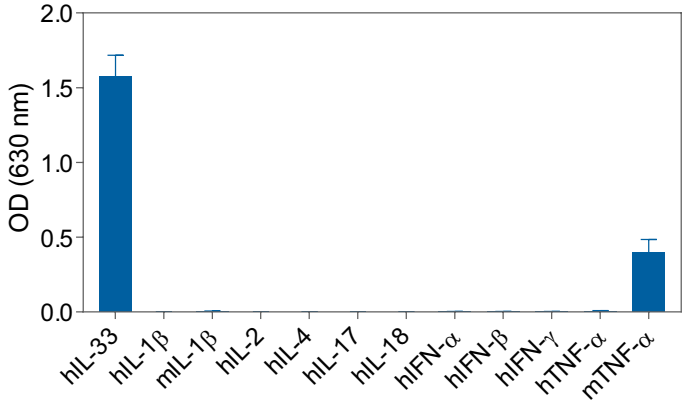

Figure 2. Response of HEK-Blue™ IL-33 cells to a panel of cytokines. Cells were stimulated with various human and murine recombinant cytokines: 1 ng/ml of hIL-33, 10 ng/ml of hIL-1β, mIL-1β, hIL-2, hIL-4, hIL-17, hIL-18, hIFN-γ, hTNF-α, mTNF-α, or 10^2 U/ml hIFN-α2a or hIFN-β. After overnight incubation, SEAP activity was assessed using QUANTI-Blue™ Solution. The OD readings at 630 nm are shown as mean ± SEM.

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