Validation data for HEK-Blue™ IL-9 Cells

https://www.invivogen.com/hek-blue-il9

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Version 22D13-NJ

HEK-Blue™ IL-9 cells allow the detection of bioactive interleukin-9 (IL-9) by monitoring STAT5 activation. These human embryonic kidney 293 (HEK293)-derived cells express the human IL-9 heterodimeric receptor (IL-9Rα and IL-2Rγ), human JAK3, human STAT5b, and a STAT5-inducible secreted embryonic alkaline phosphatase (SEAP) reporter. Human IL-9Rα expression by these cells has been verified by flow cytometry (**Figure 1**). HEK-Blue™ IL-9 cells respond to human and murine IL-9 (**Figure 2**). These cells can be used for screening of antibodies targeting the IL-9 pathway (**Figure 3**). Of note, HEK-Blue™ IL-9 cells are knock-out for human IFNAR2, and do not respond to human type I IFNs (**Figure 4**).

Validation of IL-9Rα expression

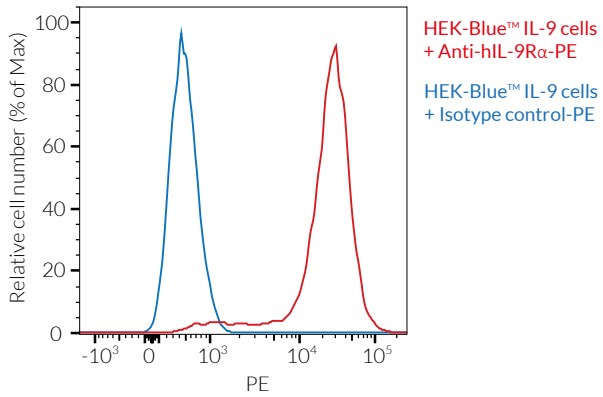

Figure 1. Validation of the expression of human IL-9Rα by HEK-Blue™ IL-9 cells. 5 x 10^5 cells were incubated with either a PE-conjugated isotype control (blue) or a PE-conjugated Anti-IL-9Rα mAb (red) for 30 minutes. The binding affinity was then measured using flow cytometry.

Cellular response to IL-9

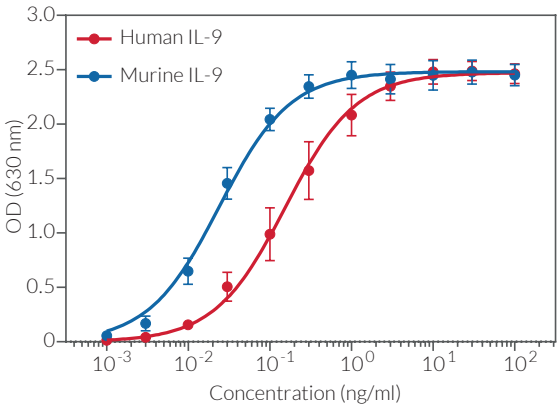

Figure 2. Dose-response of HEK-Blue™ IL-9 cells to recombinant IL-9. Cells were stimulated with increasing concentrations of recombinant human IL-9 (hIL-9) and murine IL-9 (mIL-9). After overnight incubation, the STAT5 response was determined using QUANTI-Blue™ Solution, a SEAP detection reagent. The optical density (OD) at 630 nm is shown as mean ± SEM.

IL-9 signaling inhibition

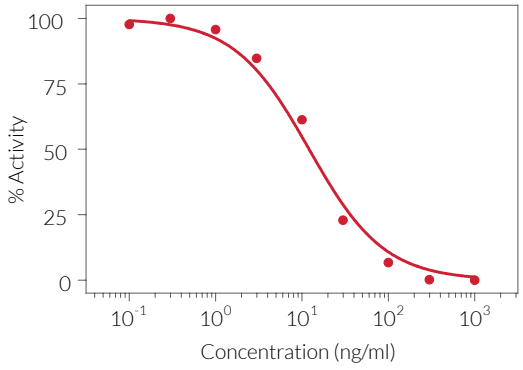

Figure 3. Dose-dependent inhibition of HEK-Blue™ IL-9 cellular response using a neutralizing antibody against hIL-9. The antibody was incubated with hIL-9 (0.3 ng/ml) for 2 hours prior to the addition of HEK-Blue™ IL-9 cells. After overnight incubation, SEAP activity in the cell culture supernatant was assessed using QUANTI-Blue™ Solution. Data are shown as a percentage (%) of activity.

Cell line specificity

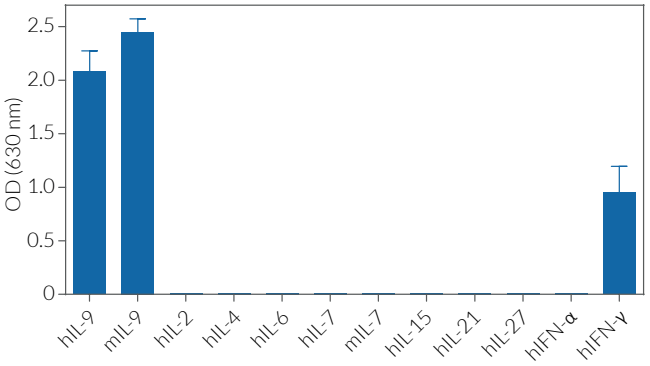

Figure 4. Response of HEK-Blue™ IL-9 cells to a panel of cytokines. Cells were stimulated with various human and murine recombinant cytokines: 1 ng/ml of hIL-9 or mIL-9, and 100 ng/ml hIL-2, hIL-4, hIL-6, hIL-7, mIL-7, hIL-15, hIL-21, or hIL-27, and 1000 U/ml hIFN-α, and 10 ng/ml hIFN-γ. After overnight incubation, SEAP activity was assessed using QUANTI-Blue™ Solution. The OD at 630 nm is shown as mean ± SEM

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