HEK-Blue™ IL-7 cells allow the detection of bioactive interleukin-7 (IL-7) by monitoring STAT5 activation. These human embryonic kidney 293 (HEK293)-derived cells express the human IL-7 heterodimeric receptor (IL-7Rα and IL-2Rγ). They also express human JAK3, human STAT5b, and a STAT5-inducible secreted embryonic alkaline phosphatase (SEAP) reporter. Human IL-7Rα expression by these cells has been verified by flow cytometry (Figure 1). HEK-Blue™ IL-7 cells respond to human and murine IL-7 (Figure 2). These cells can be used for screening of antibodies targeting the IL-7 pathway (Figure 3). Of note, HEK-Blue™ IL-7 cells are knock-out for human IFNAR2, and do not respond to human type I IFNs (Figure 4).

Validation of IL-7Rα expression

Figure 1. Validation of the expression of human IL-7Rα by HEK-Blue™ IL-7 cells. 5 x 10⁵ cells were incubated with either an APC-conjugated isotype control (blue) or an APC-conjugated Anti-IL-7Rα mAb (red) for 30 minutes. The binding affinity was then measured using flow cytometry.

Cellular response to IL-7

Figure 2. Dose-response of HEK-Blue™ IL-7 cells to recombinant IL-7. Cells were stimulated with increasing concentrations of recombinant human IL-7 (hIL-7) and murine IL-7 (mIL-7). 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-7 signaling inhibition

Figure 3. Dose-dependent inhibition of HEK-Blue™ IL-7 cellular response using a neutralizing antibody against IL-2Rγ, a subunit of IL-7R. The antibody was incubated with HEK-Blue™ IL-7 cells for 2 hours prior to the addition of hIL-7 (0.3 ng/ml). 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-7 cells to a panel of cytokines. Cells were stimulated with various human and murine recombinant cytokines: 1 ng/ml of hIL-7 or mIL-7, and 100 ng/ml hIL-2, hIL-4, hIL-6, hIL-9, mIL-9, 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.