Validation data for HEK-Blue™ CD122/CD132 cells

https://www.invivogen.com/hek-blue-il2

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Version 22A28-NJ

HEK-Blue™ CD122/CD132 cells allow the detection of bioactive human (h) IL-2 and hIL-15 by monitoring the activation of the STAT-5 pathway. IL-2 and IL-15 are closely related cytokines which share IL-2Rβ (CD122) and IL-2Rγ (CD132). HEK-Blue™ CD122/CD132 cells were generated by the stable transfection of HEK293 cells with the human IL-2Rβ (CD122) and IL-2Rγ (CD132) genes, as well as a STAT5-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. These cells can detect both hIL-2 and hIL-15, but not their murine counterparts **(Figures 1 and 2)**. They can thus be used to screen for molecules that inhibit IL-2 or IL-15 signaling such as antibodies targeting hIL-2 or hIL-15.

Interestingly, HEK-Blue™ IL-2 cells overexpressing IL-2Rα (CD25) in addition to CD122 and CD132 responds similarly to hIL-2 and hIL-15 as the HEK-Blue™ CD122/CD132 cell line **(Figure 3A and C)**. Addition of CD25 in HEK-Blue IL-2 cells does not increase the sensitivity of these cells to hIL-2, however it does increase their sensitivity to mIL-2 **(Figure 3B)**.

Cellular response to IL-2 and IL-15

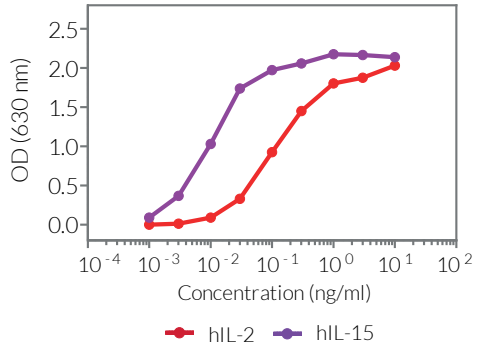

Figure 1. Dose-response of HEK-Blue™ CD122/CD132 cells to recombinant IL-2 and IL-15. Cells were stimulated with increasing concentrations of recombinant human IL-2 (hIL-2) or human IL-15 (hIL-15). After overnight incubation, the STAT5 response was determined using QUANTI-Blue™ Solution, a SEAP detection reagent, and reading the optical density (OD) at 630 nm.

Cell line specificity

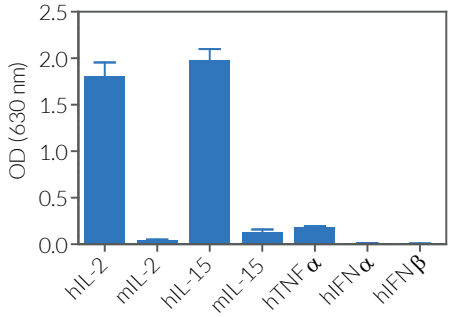

Figure 2. Cytokine response profile of HEK-Blue™ CD122/CD132 cells. Cells were stimulated with various human (h) or murine (m) recombinant cytokines; 1 ng/ml hIL-2, 1 ng/ml mIL-2, 0.1 ng/ml hIL-15, 1 ng/ml mIL-15, 10 ng/ml TNF-α, and 1x10³ IU/ml IFN-α or IFN-β. After overnight incubation, SEAP activity was assessed using QUANTI-Blue™ Solution, a SEAP detection reagent, and reading the optical density (OD) at 630 nm.

HEK-Blue™ IL-2 and HEK-Blue™ CD122/CD132 response to IL-2 and IL-15

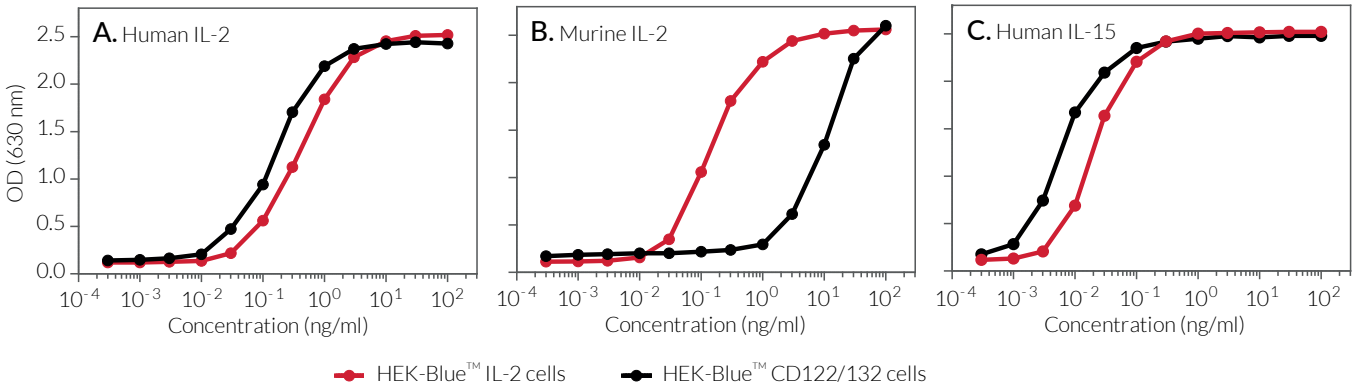

Figure 3. Dose-response of HEK-Blue™ CD122/CD132 and HEK-Blue™ IL-2 cells to recombinant IL-2 and IL-15. Cells were stimulated with increasing concentrations of recombinant human and murine IL-2, and human IL-15. After overnight incubation, the STAT5 response was determined using QUANTI-Blue™ Solution, a SEAP detection reagent, and reading the optical density (OD) at 630 nm.

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