

Validation data for HEK-Blue™ IL-5 Cells

<https://www.invivogen.com/hek-blue-il5>

For research use only

Version 24G04-NJ

HEK-Blue™ IL-5 cells allow the detection of bioactive interleukin-5 (IL-5) by monitoring STAT5 activation. These human embryonic kidney 293 (HEK293)-derived cells express the human IL-5 heterodimeric receptor (IL-5R α and IL-5R β), human STAT5b, and a STAT5-inducible secreted embryonic alkaline phosphatase (SEAP) reporter. Human IL-5R α expression by these cells has been verified by flow cytometry (Figure 1). HEK-Blue™ IL-5 cells respond to human and murine IL-5 (Figure 2). These cells can be used for screening of antibodies targeting the IL-5 pathway (Figure 3). Of note, HEK-Blue™ IL-5 cells do not respond to human type I IFNs (Figure 4).

Validation of IL-5R α expression

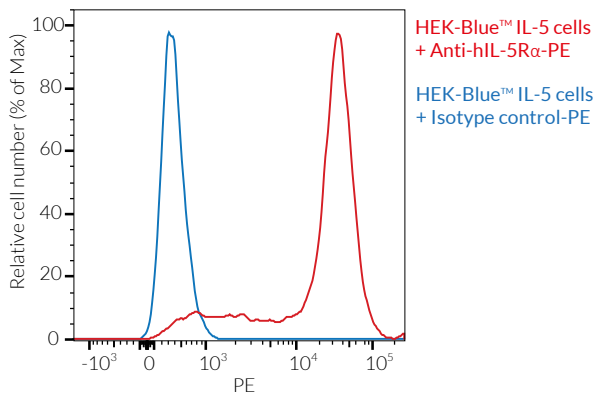


Figure 1. Validation of the expression of human IL-5R α by HEK-Blue™ IL-5 cells. 5×10^5 cells were incubated with either a PE-conjugated isotype control (blue) or a PE-conjugated Anti-IL-5R α mAb (red) for 30 minutes. The binding affinity was then measured using flow cytometry.

Cellular response to IL-5

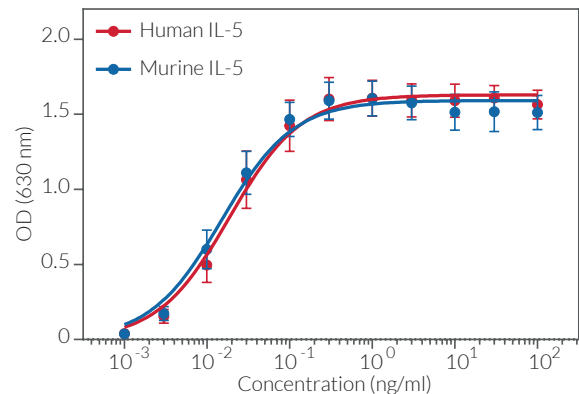


Figure 2. Dose-response of HEK-Blue™ IL-5 cells to recombinant IL-5. Cells were stimulated with increasing concentrations of recombinant human IL-5 (hIL-5) and murine IL-5 (mIL-5). After overnight incubation, the STAT5 response was determined using QUANTI-Blue™ Solution, a SEAP detection reagent. The optical density (OD) at 630 nm is shown as mean \pm SEM.

IL-5 signaling inhibition

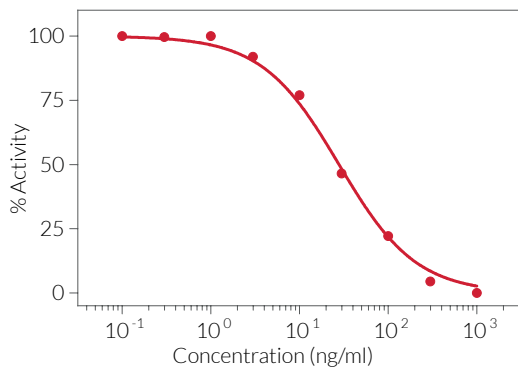


Figure 3. Dose-dependent inhibition of HEK-Blue™ IL-5 cellular response using a neutralizing antibody against IL-5. The antibody was incubated with hIL-5 (0.1 ng/ml) for 2 hours prior to the addition of HEK-Blue™ IL-5 cells. After overnight incubation, SEAP activity in the cell culture supernatant was assessed using QUANTI-Blue™ Solution. Data are shown as a percentage (%) of activity.

Cell line specificity

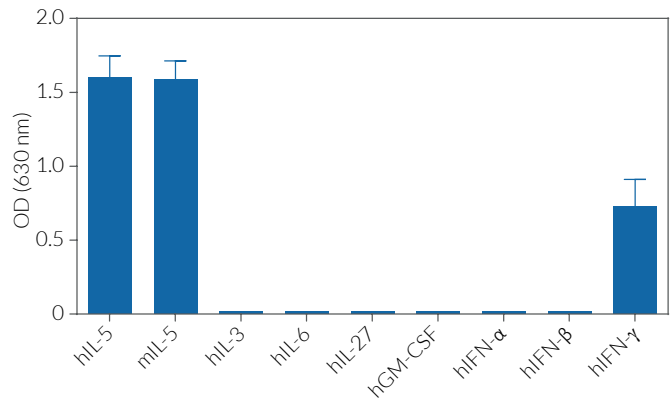


Figure 4. Response of HEK-Blue™ IL-5 cells to a panel of cytokines. Cells were stimulated with various human and murine recombinant cytokines: 0.3 ng/ml of hIL-5 or mIL-5, and 100 ng/ml hIL-3, hIL-6, hIL-27, or hGM-CSF, and 1000 U/ml hIFN- α or hIFN- β , and 10 ng/ml hIFN- γ . After overnight incubation, SEAP activity was assessed using QUANTI-Blue™ Solution. The OD at 630 nm is shown as mean \pm SEM.

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com



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