

# Validation data for HEK-Blue™ IL-36 cells

<https://www.invivogen.com/hek-blue-il36>

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Version 21J25-NJ

HEK-Blue™ IL-36 cells allow the detection of human bioactive interleukin-36 agonist isoforms (hIL-36 $\alpha$ , hIL-36 $\beta$ , and hIL-36 $\gamma$ ) by monitoring NF- $\kappa$ B/AP-1 activation. These human embryonic kidney 293 (HEK293)-derived cells express the human IL-36R (IL-1R6), the human IL-1RACP coreceptor, and a NF- $\kappa$ B/AP-1-inducible secreted embryonic alkaline phosphatase (SEAP) reporter. Human IL-36R expression by these cells has been confirmed by RT-qPCR (Figure 1). HEK-Blue™ IL-36 cells respond to human but not murine IL-36 agonist isoforms. Of note, these cells are more sensitive to hIL-36 $\alpha$  and hIL-36 $\gamma$  (detection range: 0.003-10 ng/ml) compared to hIL-36 $\beta$  (detection range: 1-10 ng/ml) (Figure 2). HEK-Blue™ IL-36 cells are also responsive to other cytokines that signal through NF- $\kappa$ B/AP-1, such as hIL-1 $\beta$  and hTNF- $\alpha$  (Figure 3).

## Validation of IL-36R expression

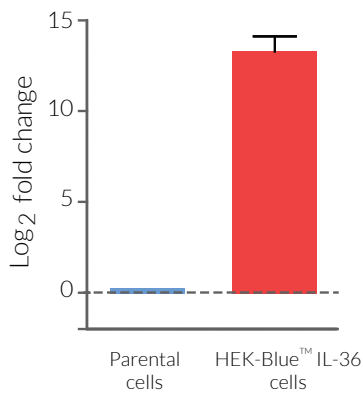


Figure 1. Human IL-36R mRNA expression in HEK-Blue™ IL-36 cells.

Total mRNA was extracted from  $\sim 1 \times 10^6$  HEK-Blue™ parental cells (blue) and HEK-Blue™ IL-36 cells (red). Human IL-36R mRNA was amplified using quantitative RT-qPCR. Data are represented as the log2 fold change comparing hIL-36R relative expression between the two cell lines.

## Cellular response to IL-36

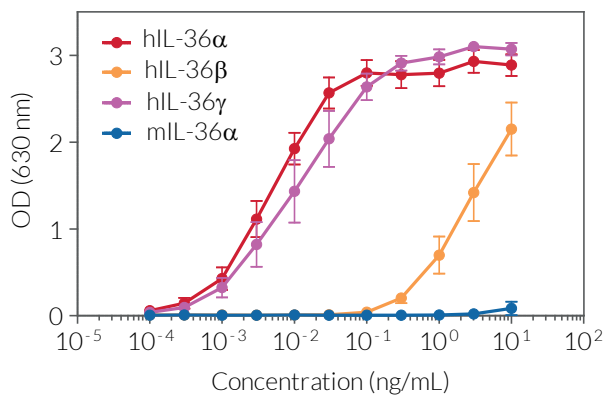


Figure 2. Dose-response of HEK-Blue™ IL-36 cells to recombinant IL-36.

Cells were stimulated with increasing concentrations of recombinant human IL-36 (hIL-36) isoforms  $\alpha$ ,  $\beta$ , or  $\gamma$  and murine IL-36 $\alpha$  (mIL-36 $\alpha$ ). After overnight incubation, the NF- $\kappa$ B response was determined using QUANTI-Blue™ Solution, a SEAP detection reagent. The optical density (OD) at 630 nm is shown as mean  $\pm$  SEM.

## Cell line specificity

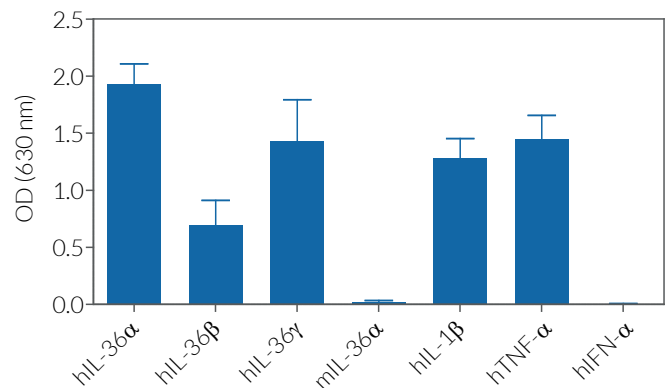


Figure 3. Response of HEK-Blue™ IL-36 cells to a panel of cytokines.

Cells were stimulated with various human and murine recombinant cytokines: 0.01 ng/ml hIL-36 $\alpha$ , 1 ng/ml hIL-36 $\beta$ , 0.01 ng/ml hIL-36 $\gamma$ , 1 ng/ml mL-36 $\alpha$ , 0.003 ng/ml hIL-1 $\beta$ , 0.1 ng/ml TNF- $\alpha$ , or 1000 U/ml hIFN- $\alpha$ . After overnight incubation, SEAP activity was assessed using QUANTI-Blue™ Solution. The optical density (OD) at 630 nm is shown as mean  $\pm$  SEM.

### TECHNICAL SUPPORT

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