

Validation data for HEK-Blue™ TPO cells

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

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Version 20L10-MM

HEK-Blue™ TPO cells allow the detection of bioactive thrombopoietin (TPO) by monitoring STAT5 activation. These human embryonic kidney 293 (HEK293)-derived cells express the human TPO receptor (TPO-R), human STAT5 and a STAT5-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. Human TPO-R surface expression by these cells has been verified by flow cytometry (figure 1) and human STAT5 expression has been confirmed by RT-qPCR (data not shown). HEK-Blue™ TPO cells respond to both human TPO (detection range: 3-100 ng/ml; figure 2) and to murine TPO (detection range: 0.1-10 ng/ml). Of note, as HEK293 cells endogenously express the receptors for type I and type III interferons (IFNs), these cells respond to IFN- α/β and IFN- γ (figure 3). HEK-Blue™ TPO cells are non-responsive to human IL-2 (hIL-2) and hIL-15 (figure 3).

Validation of TPO-R surface expression by FACS

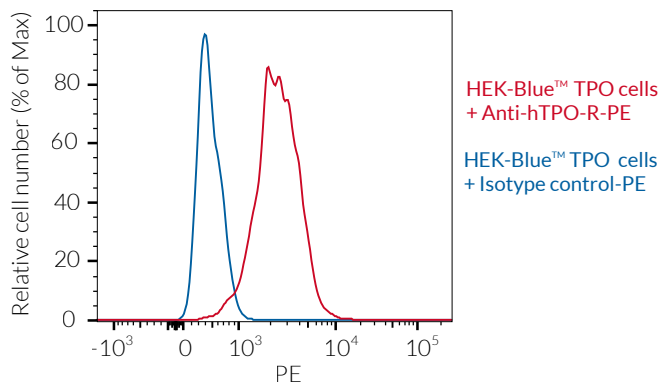


Figure 1. Surface expression of human TPO-R by HEK-Blue™ TPO cells. $\sim 2 \times 10^5$ HEK-Blue™ TPO cells were incubated with either a PE-conjugated Anti-hTPO-R mAb or a PE-conjugated mouse IgG2b isotype control for 1 hour. Cell surface staining was analyzed by flow cytometry.

Cellular response to TPO

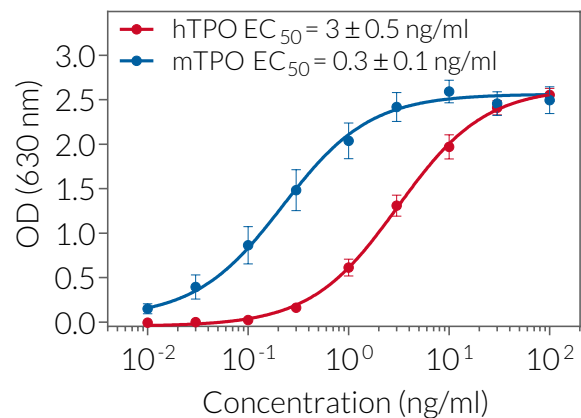


Figure 2. Dose-response of HEK-Blue™ TPO cells to recombinant TPO. Cells were stimulated with increasing concentrations of recombinant human TPO (hTPO) and murine TPO (mTPO). After overnight incubation, the STAT5 response was determined using QUANTI-Blue™ Solution, a SEAP detection reagent, and reading the optical density (OD) at 630 nm. EC_{50} values are shown as mean \pm SEM.

Cell line specificity

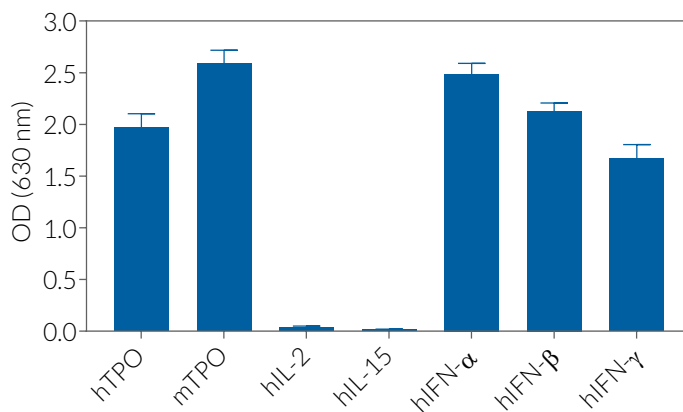


Figure 3. Response of HEK-Blue™ TPO cells to a panel of cytokines. Cells were stimulated with various human and murine recombinant cytokines: 10 ng/ml of hTPO, mTPO, hIL-2, hIL-15, hIFN- γ , or 30 U/ml hIFN- α or hIFN- β . After overnight incubation, SEAP activity was assessed using QUANTI-Blue™ Solution. The optical density (OD) at 630 nm is shown as mean \pm SEM.

TECHNICAL SUPPORT

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