

Validation data for HEK-Blue™ TPO cells

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Version 25L11-NJ

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 expression by these cells has been verified by flow-cytometry (**Figure 1**). HEK-Blue™ TPO cells respond to both human and mouse TPO (**Figure 2**). Of note, as HEK293 cells endogenously express the interferon- α/β receptor (IFNAR), these cells respond to type I IFNs. They also respond to IFN- γ (**Figure 3**). However, HEK-Blue™ TPO cells are non-responsive to human IL-2 (hIL-2) and hIL-15, two cytokines that also signal through STAT5 (**Figure 3**).

Validation of TPO-R surface expression

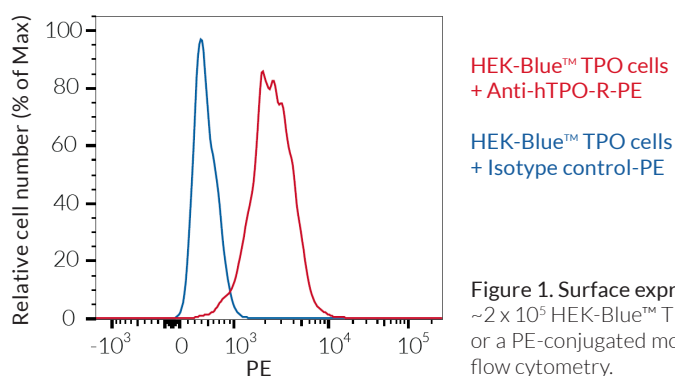


Figure 1. Surface expression of human TPO-R by HEK-Blue™ TPO cells.

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

Cellular response to TPO

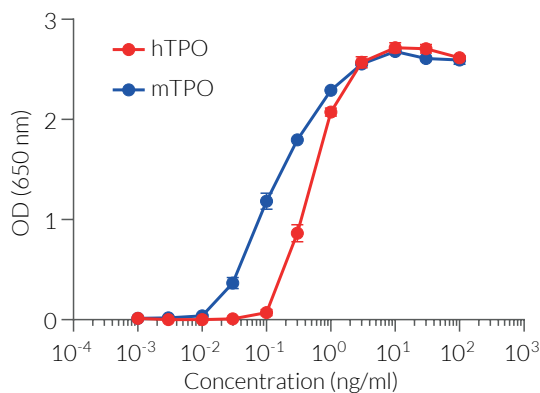


Figure 2. Dose-response of HEK-Blue™ TPO cells to recombinant TPO. Cells were stimulated with increasing concentrations of recombinant (h) TPO (cat code: rcyc-htpo) and mouse (m) TPO. After overnight incubation, the STAT5 response was determined using QUANTI-Blue™ Solution, a SEAP detection reagent. The optical density (OD) at 650 nm is shown as mean \pm SEM.

Cell line specificity

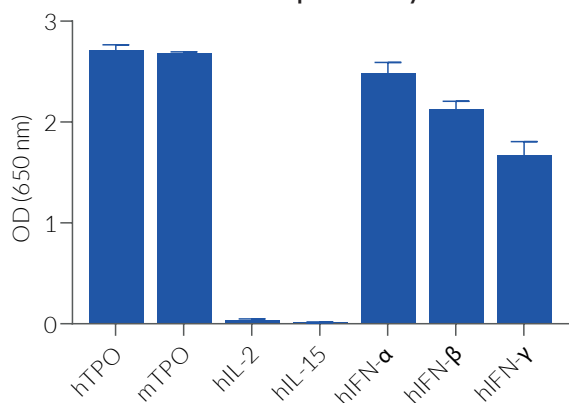


Figure 3. Response of HEK-Blue™ TPO cells to a panel of cytokines. Cells were stimulated with various human (h) and mouse (m) recombinant cytokines: 10 ng/ml of hTPO (cat code: rcyc-htpo), mTPO, hIL-2, hIL-15, hIFN- γ , or 30 U/ml hIFN- α or hIFN- β . After overnight incubation, SEAP activity was assessed using QUANTI-Blue™ Solution. The optical density (OD) at 650 nm is shown as mean \pm SEM.

TECHNICAL SUPPORT

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