

# Validation data for HEK-Blue™ RANKL cells

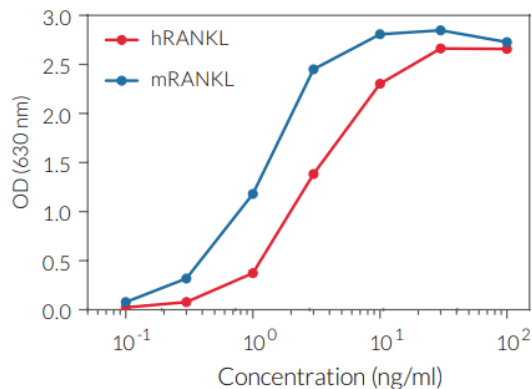
<https://www.invivogen.com/hek-blue-rankl>

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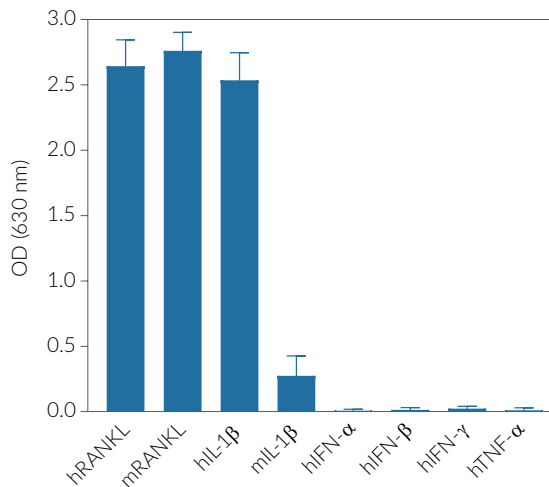
HEK-Blue™ RANKL cells allow the detection of human (h) and murine (m) RANKL by monitoring NF-κB and AP-1 activation. These human embryonic kidney 293 (HEK293)-derived cells express the human RANKL receptor, RANK, and an NF-κB- and AP-1-inducible secreted embryonic alkaline phosphatase (SEAP) reporter. HEK-Blue™ RANKL cells respond to hRANKL and mRANKL (figures 1 & 2). Of note, these cells also respond to hIL-1β and, to a weaker extent, mL-1β, as they endogenously express the IL-1β receptor. They are not responsive to human IFN-α, IFN-β, IFN-γ, and TNF-α (figure 2). HEK-Blue™ RANKL cells can be used for screening antibodies targeting the RANKL/RANK pathway (figure 3).

## Cellular response to RANKL



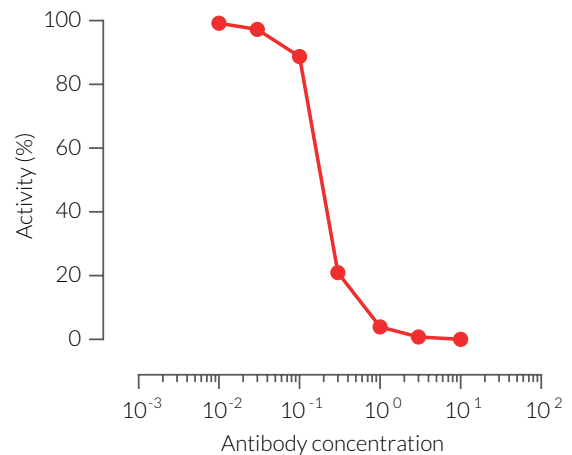
**Figure 1.** Dose-response of HEK-Blue™ RANKL cells to recombinant RANKL. Cells were stimulated with increasing concentrations of recombinant human or murine RANKL. After overnight incubation, the NF-κB response was determined using QUANTI-Blue™ Solution, a SEAP detection reagent, and reading the optical density (OD) at 630 nm.

## Cell line specificity



**Figure 2.** HEK-Blue™ RANKL cell responses to cytokines. Cells were stimulated with 10 ng/ml of hRANKL, mRANKL, hIL-1β, mL-1β, 1x10<sup>4</sup> IU/ml of hIFN-α, hIFN-β, 100 ng/ml of hIFN-γ, or hTNF-α. After overnight incubation, SEAP activity was assessed using QUANTI-Blue™ Solution. The optical density (OD) at 630 nm is shown as mean ± SEM.

## Human RANKL signaling inhibition



**Figure 3.** Dose-dependent inhibition of HEK-Blue™ RANKL cells response using denosumab biosimilar. A serial dilution of denosumab, a biosimilar Anti-hRANKL-hIgG2 monoclonal antibody (mAb) was incubated with 10 ng/ml of recombinant human RANKL for 2 hours prior to the addition of the HEK-Blue™ RANKL cells. After overnight incubation, the NF-κB response was determined using QUANTI-Blue™ Solution, a SEAP detection reagent. Data are presented as percentage of activity.

### TECHNICAL SUPPORT

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