Validation data for HEK-Blue™ RANKL cells

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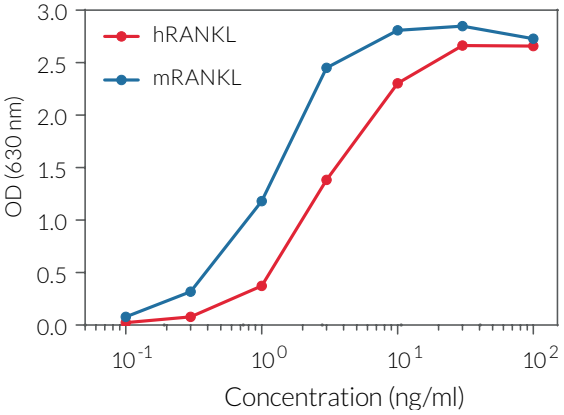
Version 19G11-NJ

HEK-Blue™ RANKL cells allow the detection of human (hRANKL) and murine RANKL (mRANKL) by monitoring NF-κB and AP-1 activation. They were generated by stable transfection of HEK293 cells with the genes encoding the receptor activator of NF-κB (RANK), and an NF-κB- and AP-1-inducible SEAP (secreted embryonic alkaline phosphatase) reporter. The sensitivity of this cell line to hRANKL and mRANKL has been determined and ranges from 1 ng/ml to 100 ng/ml (**figures 1 & 2**). Of note, as HEK293 and HEK-Blue™ RANKL cells endogenously express the IL-1β receptor, they display a strong response to hIL-1β and a weak response to mL-1β (**figure 2**). These cells feature a triple knockout of TLR3, TLR5 and TNFR (which are endogenously expressed in HEK293 cells) and hence do not respond to TLR3 and TLR5 ligands, nor to TNF-α. HEK-Blue™ RANKL cells can also be used for the screening of anti-RANKL antibodies (**figure 3**).

Evaluation of cellular response to RANKL



Cytokine	EC_{50} (ng/ml)
hRANKL	3.0 ± 1.5
mRANKL	1.2 ± 0.5

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. EC_{50} values are shown as mean ± SD.

Cytokine and TLR responses

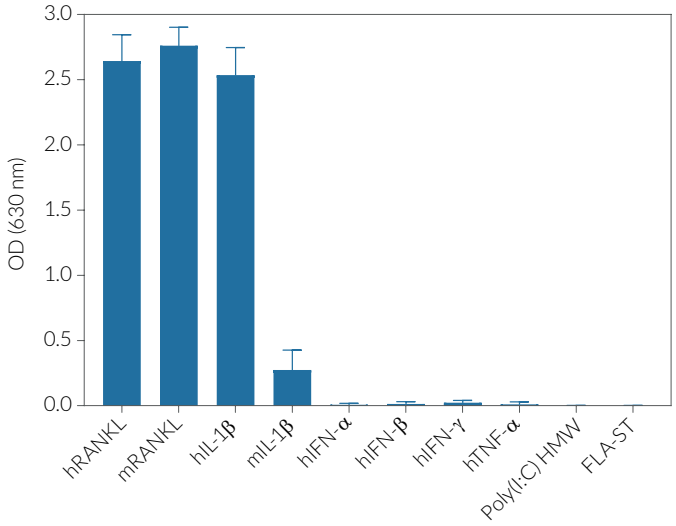


Figure 2. HEK-Blue™ RANKL cell responses to cytokines and TLR3 and TLR5 agonists. Cells were stimulated with 10 ng/ml of hRANKL, mRANKL, hIL-1β, mIL-1β, $1x10^4$ IU/ml of hIFN-α, hIFN-β, 100 ng/ml of hIFN-γ, hTNF-α, 1 µg/ml of Poly(I:C) HMW (a TLR3 agonist) or FLA-ST (flagellin from *S. typhimurium*) (a TLR5 agonist). After overnight incubation, SEAP activity was assessed using QUANTI-Blue™ Solution and reading the optical density (OD) at 630 nm.

Evaluation of RANKL/RANK signaling inhibition

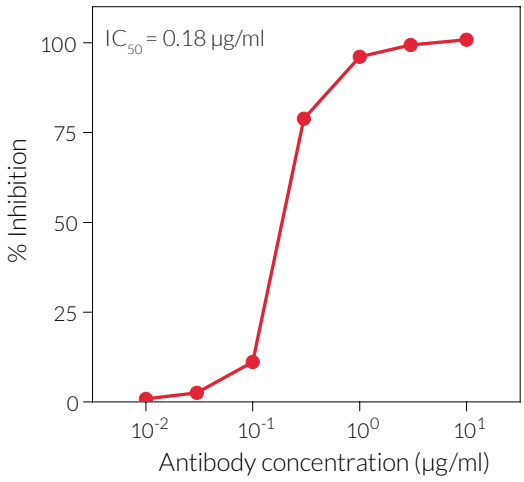


Figure 3. Dose-dependent inhibition of HEK-Blue™ RANKL cellular response to RANKL using an anti-hRANKL antibody. The antibody was incubated with HEK-Blue™ RANKL cells for 30 minutes prior to the addition of 10 ng/ml of hRANKL. After overnight incubation, SEAP activity in the cell culture supernatant was assessed using QUANTI-Blue™ Solution and reading the optical density (OD) at 630 nm. Data represent % inhibition of reporter activity without anti-hRANKL antibody.

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