

Validation data for Anti-hRANKL-hIgG2

<https://www.invivogen.com/anti-human-rankl-denosumab>

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Version 24K06-AK

Anti-hRANKL-hIgG2 is the biosimilar of the clinical antibody denosumab. It is a neutralizing monoclonal antibody (mAb) against the human receptor activator of nuclear factor- κ B ligand (hRANKL). Anti-hRANKL-hIgG2 features a human IgG2 constant region (Fc) and the variable region of denosumab which targets the transmembrane and soluble forms of the human RANKL antigen. The binding capacity of Anti-hRANKL-hIgG2 to the hRANKL antigen has been validated by ELISA, using coated hRANKL protein and a Anti-hIgG-HRP secondary antibody (**Figure 1**). The ability of Anti-hRANKL-hIgG2 biosimilar denosumab to neutralize the biological activity of hRANKL has been confirmed using InvivoGen's HEK-Blue™ RANKL cells (**Figure 2**).

Validation of Anti-hRANKL-hIgG2 mAb by ELISA

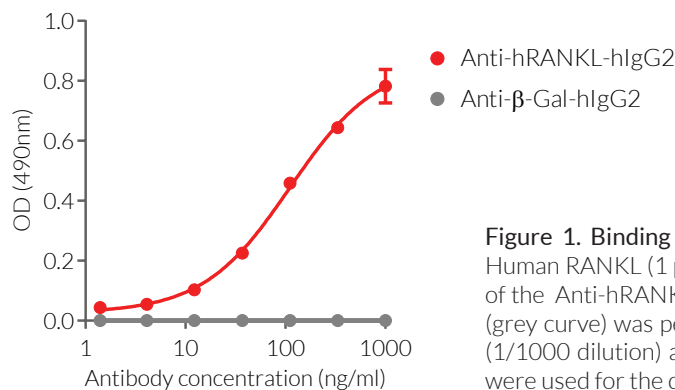


Figure 1. Binding of Anti-hRANKL-hIgG2 mAb to coated human RANKL protein. Human RANKL (1 μ g/ml) was coated on ELISA plates overnight. A 3-fold serial dilution of the Anti-hRANKL-hIgG2 mAb (red curve) or the Anti- β -Gal-hIgG2 control antibody (grey curve) was performed for the capture step. An HRP-labelled anti-hIgG antibody (1/1000 dilution) and the HRP substrate OPD (o-phenylenediamine dihydrochloride) were used for the detection step. Absorbance was read at 490 nm.

Dose-dependent neutralization of RANK/RANKL signaling

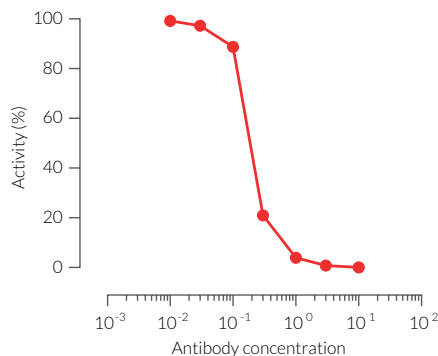


Figure 2. Dose-dependent inhibition of HEK-Blue™ RANKL cells response using denosumab biosimilar. A serial dilution of denosumab, a biosimilar Anti-hRANKL-hIgG2 monoclonal antibody (mAb) was incubated with 10 ng/ml of recombinant human RANKL for 2 hours prior to the addition of the HEK-Blue™ RANKL cells.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

TECHNICAL SUPPORT

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