

Validation data for Anti-hRANKL-hIgG2

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

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Version 23L06-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 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

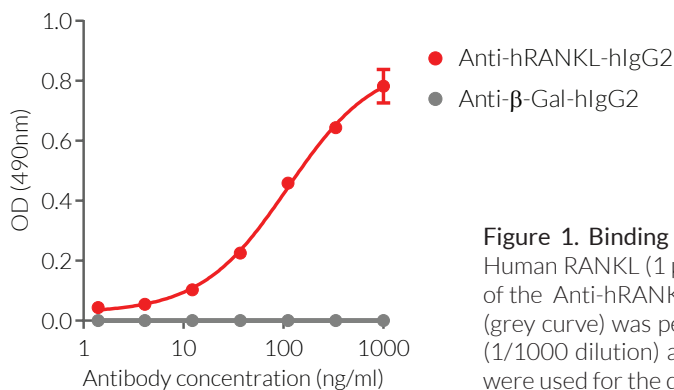


Figure 1. Binding of Anti-hRANKL-hIgG2 mAb to coated human RANKL protein. Human RANKL (1 μ g/ml) was coated on ELISA plates overnight. A 3-fold serial dilution of the Anti-hRANKL-hIgG2 mAb (red curve) or the Anti- β -Gal-hIgG2 control antibody (grey curve) was performed for the capture step. An HRP-labelled 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

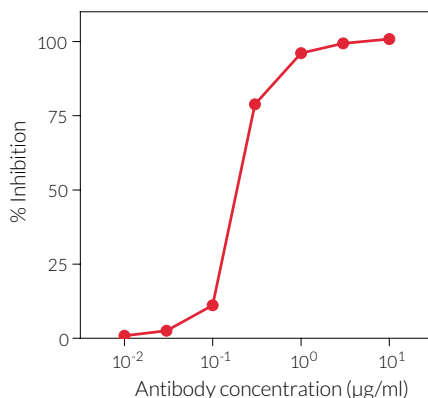


Figure 2. Dose-dependent inhibition of HEK-Blue™ RANKL cellular response using the Anti-hRANKL-hIgG2 antibody. Increasing concentration of the Anti-hRANKL-hIgG2 antibody was incubated with HEK-Blue™ RANKL cells for 30 minutes before the addition of 10 ng/ml of hRANKL. After overnight incubation, SEAP activity in the cell culture supernatant was assessed using QUANTI-Blue™ Solution. Data represent % inhibition of reporter activity without anti-hRANKL antibody.

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com