

BioSim™ Denosumab (Prolia®)(Human) ELISA Kit

rev 11/17

(Catalog # E4380-100, 100 assays, Store at 4°C)

I. Introduction:

Denosumab (Prolia®) is a human monoclonal antibody that binds to RANKL, a transmembrane or soluble protein essential for the formation, function, and survival of osteoclasts, the cells responsible for bone resorption. Prolia prevents RANKL from activating its receptor, RANK, on the surface of osteoclasts and their precursors. Prevention of the RANKL/RANK interaction inhibits osteoclast formation, function, and survival, thereby decreasing bone resorption and increasing bone mass and strength in both cortical and trabecular bone. BioSim™ Denosumab ELISA kit has been developed for specific quantification of Denosumab concentration in human serum or plasma with high sensitivity and reproducibility.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Denosumab.

Detection Range: 3 - 300 ng/ml

Sensitivity: 3 ng/ml

Assay Precision: Intra-Assay: CV < 15%; Inter-Assay: CV < 15% (CV (%) = SD/mean X 100)

Recovery rate: 85 – 115% with normal human serum samples with known concentrations

Cross Reactivity: There is no cross reaction with native serum immunoglobulins and tested monoclonal antibodies such as infliximab (Remicade®), adalimumab (Humira®), trastuzumab (Herceptin®) and bevacizumab (Avastin®).

III. Sample Type:

Human serum and plasma

IV. Kit Contents:

Components	E4380-100	Part No.
Micro ELISA Plate	1 plate	E4380-100-1
Denosumab Standards (S1 – S8)	1 ml X 8	E4380-100-2.x
Assay Buffer	50 ml	E4380-100-3
HRP-conjugate Probe	12 ml	E4380-100-4
TMB substrate (Avoid light)	12 ml	E4380-100-5
Stop Solution	12 ml	E4380-100-6
Wash buffer (20X)	50 ml	E4380-100-7
Plate sealers	2	E4380-100-8

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Calibrated measures
- Precision pipettes with disposable tips
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment.

VII. Reagent and Sample Preparation:

Note: Prepare reagents within 30 minutes before the experiment.

Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Wash Buffer:** Dilute the 20X Wash Buffer to 1X solution in ddH₂O (10 ml of Wash Buffer stock to 190 ml of ddH₂O). Mix the 1X solution thoroughly by vortex manually. The working stock can be stable for 2 weeks after preparation at 4°C.

2. Standard Preparation:

Ready to use

Name	S1	S2	S3	S4	S5	S6	S7	S8
Conc. (ng/ml)	300	100	30	10	3	0	High Control	Low Control

3. Sample Dilution:

- **Serum/Plasma:** Dilute samples 1:50 (10 µl Sample + 490 µl Assay Buffer).
- Diluted samples should further be diluted if the concentration of Denosumab is higher than the measuring range.
- The usual precautions for venipuncture should be observed. Samples are stable at 4°C for 2 days and -20°C for 6 months. Avoid freeze-and-thaw cycle.

VIII. Assay Protocol:

Note: Bring all reagents, microplate and samples to room temperature 15 minutes prior to the assay.

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It is recommended that all standards and samples be run at least in duplicate.
 A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Add 100 μ l of **standards, controls** and **diluted-samples** into appropriate wells. Cover wells and incubate for 30 minutes at room temperature (RT).
3. Discard incubation solution. Wash plate 3 times each with 300 μ l of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
4. Add 100 μ l of **HRP-conjugate** into each well. Cover wells with adhesive plate sealer and incubate at RT for 30 minutes.
5. Discard the solution and wash the wells as step 3.
6. Add 100 μ l of 1X **TMB substrate** solution and incubate the plate in dark at RT for 10 minutes
7. Add 100 μ l of **Stop solution** to stop the reaction
8. Read the absorbance in micro plate reader set to 450 nm within 20 minutes. (reference wavelength to 650 nm)

IX. CALCULATION:

Using the standards disregarding zero standard, construct a standard curve by plotting the OD_{450/650} nm for each of 5 standards on the Y-axis versus the corresponding Denosumab concentration on the X-axis. Construct a standard curve of difference data using software capable of generating four parameter logistic (4PL) or point-to-point calculation curve fit. To obtain the exact values of the samples, the concentration determined from the standard-curve should be multiplied by the dilution factor.

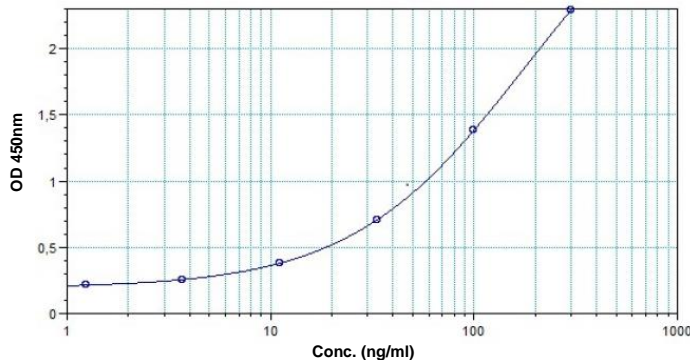


Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

X. RELATED PRODUCTS:

- BioSim™ Rituximab (Mabthera®) (Human) ELISA Kit (Cat. No. E4371-100)
- BioSim™ Adalimumab (Humira®) (Human) ELISA Kit (Cat. No. E4372-100)
- BioSim™ Bevacizumab (Avastin®) (Human) ELISA Kit (Cat. No. E4373-100)
- BioSim™ Etanercept (Enbrel®) (Human) ELISA Kit (Cat. No. E4374-100)
- BioSim™ Ipilimumab (Remicade®) (Human) ELISA Kit (Cat. No. E4375-100)
- BioSim™ Trastuzumab(Herceptin®)(Human) ELISA Kit (Cat. No. E4376-100)
- BioSim™ Golimumab (Simponi®)(Human) ELISA Kit (Cat. No. E4377-100)
- BioSim™ Infliximab (Remsima®)(Human) ELISA Kit (Cat. No. E4378-100)
- BioSim™ Cetuximab (Erbix®)(Human) ELISA Kit (Cat. No. E4379-100)
- BioSim™ Denosumab (Prolia®)(Human) ELISA Kit (Cat. No. E4380-100)
- BioSim™ Omalizumab (Xolair®)(Human) ELISA Kit (Cat. No. E4381-100)
- BioSim™ Nivolumab (Opdivo®)(Human) ELISA Kit (Cat. No. E4382-100)
- BioSim™ Pembrolizumab (Keytruda®)(Human) ELISA Kit (Cat. No. E4383-100)
- BioSim™ Ipilimumab (Yervoy®)(Human) ELISA Kit (Cat. No. E4384-100)

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