

Version 3a Last updated 17 April 2023

# **ab237649**

## **Denosumab ELISA Kit**

For the measurement of Denosumab in human serum and plasma.

This product is for research use only and is not intended for diagnostic use.

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# 1. Overview

Denosumab ELISA kit (ab237649) has been developed for specific quantification of Denosumab concentration in human serum or plasma with high sensitivity and reproducibility. The density of color is proportional to the amount of Denosumab captured from the samples and can be quantified when compared with standard curve.

Denosumab 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.

## 2. Protocol Summary

Prepare all reagents, samples, and standards as instructed



Add 100  $\mu$ L standard or sample to appropriate wells. Cover and incubate for 30 minutes at room temperature



Discard incubation solution and wash plate 3 times with 300  $\mu$ L diluted Wash Buffer



Add 100  $\mu$ L HRP-conjugate to each well. Cover and incubate for 30 minutes at room temperature



Discard the solution and wash plate 3 times with 300  $\mu$ L diluted Wash Buffer



Add 100  $\mu$ L TMB Substrate and incubate the plate in the dark at room temperature for 10 minutes.



Add 100  $\mu$ L Stop Solution and read OD at 450 nm within 20 minutes.

### 3. Precautions

Please read these instructions carefully prior to beginning the assay.

- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:  
[www.abcam.com/assaykitguidelines](http://www.abcam.com/assaykitguidelines)
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

### 4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components.

### 5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors.

### 6. Materials Supplied

Item	Quantity	Storage
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		<b>condition</b>
Micro ELISA Plate	1 unit	+4°C
Denosumab Standard S1	1 mL	+4°C
Denosumab Standard S2	1 mL	+4°C
Denosumab Standard S3	1 mL	+4°C
Denosumab Standard S4	1 mL	+4°C
Denosumab Standard S5	1 mL	+4°C
Denosumab Standard S6	1 mL	+4°C
Denosumab Standard S7	1 mL	+4°C
Assay Buffer	50 mL	+4°C
HRP-conjugate Probe	12 mL	+4°C
TMB Substrate	12 mL	+4°C
Stop Solution	12 mL	+4°C
Wash Buffer (20X)	50 mL	+4°C
Plate sealers	2 units	+4°C

## 7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 450 nm
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for sample dilution.
- Plate shaker for all incubation steps.
- Absorbent paper

## 8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.

## 9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

### 9.1 20X Wash Buffer:

Dilute the 20X Wash Buffer to 1X solution in ddH<sub>2</sub>O (10 mL of Wash Buffer stock to 190 mL of ddH<sub>2</sub>O). Mix the 1X solution thoroughly by vortex manually. The working stock can be stable for 2 weeks after preparation at 4°C.

## 10. Standard and Control Preparation

Standard and controls, S1 – S7, are ready to use, please see table below for concentrations:

Name	S1	S2	S3	S4	S5	S6	S7
Conc. ng/mL	300	100	30	10	0	High control	Low Control

Concentration for high and low controls are indicated on vials.



## 11. Sample Preparation

### General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples for the most reproducible assay.

#### 11.1 Serum/plasma:

1. Dilute samples at 1:50 (10 µL serum/plasma + 490 µL Assay buffer).
2. Diluted samples should further be diluted if the concentration of Denosumab is higher than the measuring range.
3. Samples are stable at 4°C for 2 days and -20°C for 6 months. Avoid freeze-and-thaw cycle.

**Δ Note:** The usual precautions for venipuncture should be observed.

## 12. Assay Procedure

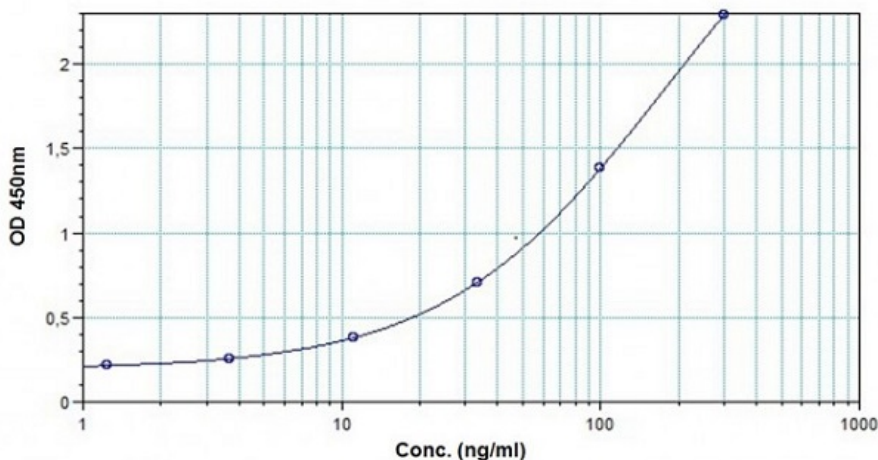
- Prepare reagents within 30 minutes before the experiment.
  - Equilibrate all materials and prepared reagents to room temperature 15 minutes prior to use.
  - We recommend that you assay all standards, controls and samples in duplicate.
- 
- 12.1** Add 100 µL of standards, controls and diluted samples into appropriate wells. Cover wells and incubate for 30 minutes at room temperature.
  - 12.2** Discard incubation solution. Wash plate 3 times each with 300 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.
  - 12.3** Add 100 µL of HRP-conjugate into each well. Cover wells with adhesive plate sealer and incubate at room temperature for 30 minutes.
  - 12.4** Discard the solution and wash the wells as step 12.2.
  - 12.5** Add 100 µL of 1X TMB substrate solution and incubate the plate in the dark at room temperature for 10 minutes.
  - 12.6** Add 100 µL of Stop solution to stop the reaction.
  - 12.7** Read the absorbance in a microplate reader set to 450 nm within 20 minutes. (Reference wavelength to 650 nm).

## 13. Calculations

- 13.1 Calculate the average absorbance value for the blank control (0 ng/mL) standards. Subtract the average blank control standard absorbance value from all other absorbance values.
- 13.2 **Create a standard curve** by plotting the average blank control subtracted absorbance value for each standard concentration (y axis) against the target protein concentration (x axis) of the standard.
- 13.3 Construct a standard curve of difference data using software capable of generating four-parameter logistic (4PL) or point-to-point calculation curve fit.
- 13.4 To obtain the exact values of the samples, the concentration determined from the standard curve should be multiplied by the dilution factor.
- 13.5 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted **absorbance values against the standard curve**. Multiply the resulting value by the appropriate dilution factor to obtain the concentration of target protein in the sample.
- 13.6 Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

## 14. Typical Data

Typical standard curve - data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.



**Figure 1.** Typical Standard Curve: This standard curve is for demonstration only. A standard curve must be run with each assay.

## 15. Typical Sample Values

**Detection Range:** 10 - 300 ng/mL.

**Sensitivity:** 10 ng/mL.

**Assay Precision:** Intra-Assay: CV < 30%; Inter-Assay: CV < 30% (CV (%) = SD/mean X 100)

**Cross Reactivity:** There is no cross reaction with native serum immunoglobulins and tested monoclonal antibodies such as infliximab, adalimumab, trastuzumab and bevacizumab.

**Recovery rate:** <100±30% with normal human serum samples with known concentrations.

## 16. Notes





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