Version 11-05/24

User's Manual

Denosumab ELISA Kit

REF **DEIASL110**

Σ **96T**

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Denosumab ELISA is used as an analytical tool for quantitative determination of Denosumab in serum, plasma and cell culture supernatant.

General Description

Denosumab (trade names Prolia and Xgeva) is a human monoclonal antibody for the treatment of osteoporosis, treatment-induced bone loss, metastases to bone, and giant cell tumor of bone. Denosumab is contraindicated in people with low blood calcium levels. The most common side effects are joint and muscle pain in the arms or legs. Denosumab is a RANKL inhibitor, which works by preventing the development of osteoclasts which are cells that break down bone (bone resorption).

Principles of Testing

The method employs the quantitative sandwich enzyme immunoassay technique. Antibodies to Denosumab are pre-coated onto microwells. Samples and standards are pipetted into microwells and human Denosumab present in the sample are bound by the capture antibody. Then, a HRP (horseradish peroxidase) conjugated anti-Denosumab antibody is pipetted and incubated. After washing microwells in order to remove any non-specific binding, the ready to use substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Denosumab in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Reagents And Materials Provided

1. Anti-Denosumab Coated Microtiter Plate (12x8 wells) – 1 no
2. Denosumab Standard, (0.5 ml/vial) – 0, 2.5, 5, 10, 20, 40, 80 and 160 ng/ml
3. Anti-Denosumab:HRP Conjugate – 12 ml
4. Assay Diluent – 6 ml
5. Sample Diluent – 50 ml
6. Wash Buffer (20X) – 25 ml
7. TMB Substrate – 12 ml
8. Stop Solution – 12 ml
9. Instruction Manual

Materials Required But Not Supplied

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25µl to 1000µl
3. Deionized (DI) water

4. Wash bottle or automated microplate washer
5. Semi-Log graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Storage

1. All reagents should be stored at 2°C to 8°C for stability.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25°C). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Specimen Collection And Preparation

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20°C.

For Cell Culture Supernatant – If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at -20°C or -80ºC. Avoid repeated freeze-thaw cycles.

Test Sample preparation - Samples have to be diluted 1:500 to 1:1000 (v/v), e.g. for 1:500 (1 µl sample + 499 µl sample diluent) prior to assay. The samples may be kept at 2 - 8°C for up to three days. Long-term storage requires -20°C.

Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

Reagent Preparation

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make Wash Buffer (1X); dilute 50 ml of 20X Wash Buffer in 950 ml of DI water.

Assay Procedure

1. It is strongly recommended that all Controls and Samples be run in duplicates or triplicates. A standard curve is required for each assay. All steps must be performed at 37°C.
2. Pipette out 50 µl of Assay Diluent in each well.
3. Add 100 µl of Standards or Samples into the respective wells.

Quality Control

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Calculation

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Semi-Log graph paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Denosumab concentrations, find the unknown's Mean Absorbance value on the Yaxis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the Xaxis and read the Denosumab Concentration. If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit is best recommended for automated results.

Note:

It is recommended to repeat the assay at a different dilution factor in the following cases:

- If the sample absorbance value is below the first standard.

- If the absorbance value is equivalent or higher than the 160 ng/ml standard.

Precision

Precision is defined as the percent coefficient of variation (%CV) i.e. standard deviation divided by the mean and multiplied by 100. Assay precision was determined by both intra (n=5 assays) and inter assay (n=5 assays) reproducibility on two pools with low (2.5ng/ml), medium (20ng/ml) and high (160ng/ml) concentrations. While actual precision may vary from laboratory to laboratory and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results.

Pool	Intra Assay %CV	Inter Assay %CV
Low	<10%	<10%
Medium	<5%	<5%
High	<5%	<5%

Detection Limit

It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD. 10 replicates of '0' standards were evaluated and the LOD was found to be less than 2.5ng/ml

Linearity

Standards provided in the kit will be used for measuring the linearity range of Denosumab present in matrix.

Precautions

1. This kit is for research use only. Follow the working instructions carefully.
2. The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents

3. Do not use or mix reagents from different lots.
4. Do not use reagents from other manufacturers.
5. Avoid time shift during pipetting of reagents.
6. All reagents should be kept in the original shipping container.
7. Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
8. Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
9. Since the kit contains potentially hazardous materials, the following precautions should be observed
10. Do not smoke, eat or drink while handling kit material
11. Always use protective gloves
12. Never pipette material by mouth
13. Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
14. In any case GLP should be applied with all general and individual regulations to the use of this kit.

Limitations

Healthy individuals should be tested negative by the Denosumab. This kit is for research use only and should not be used as a diagnostic tool.