Version 11-04/24

User's Manual

Anti-Teriparatide ELISA Kit

REF **DEIAZ0072**

Σ **96T**

RUO

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

General Description

Teriparatide is a recombinant protein form of parathyroid hormone consisting of the first (N-terminus) 34 amino acids, which is the bioactive portion of the hormone. It is an effective anabolic (i.e., bone growing) agent used in the treatment of some forms of osteoporosis. The Teriparatide ELISA kit is designed to measure teriparatide with high specificity while minimizing cross-reactivity to human PTH (1-84) and other PTHrPs

Principles of Testing

The method employs the quantitative sandwich enzyme immunoassay technique. Teriparatide is pre-coated onto microwells. Samples and standards are pipetted into microwells and antibodies to Teriparatide present in the sample are bound by the capture antibody. Then, a HRP (horseradish peroxidase) conjugated Teriparatide 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 Anti-Teriparatide in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Reagents And Materials Provided

1. Teriparatide Coated Microtiter Plate (12×8 wells)
2. Anti-Teriparatide Standard, (0.5 ml/vial): 0, 10, 20, 40, 80, 160, 320 and 640 ng/ml
3. Teriparatide:HRP Conjugate, 6 ml
4. Assay Diluent, 6 ml
5. Sample Diluent, 50 ml
6. (20×) Wash Buffer, 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 25 µl to 1000 µl
3. Deionized (DI) water

4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Storage

1. All the reagents and wash solutions should be used within 12 months from manufacturing date.
2. 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.
3. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Specimen Collection And Preparation

Sample Storage:

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 -80°C. Avoid repeated freeze-thaw cycles.

Preparation Before Use:

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

Serum and Plasma Test Sample preparation: Samples have to be diluted 1:10 to 1:100 (v/v), e.g. for 1:100 (5 µl sample + 495 µl sample diluent) prior to assay. The samples may be kept at 2-8°C for up to three days. Longterm storage requires -20°C.

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 (1×); dilute 25 ml of 20× Wash Buffer in 475 ml of DI water.

Assay Procedure

Note:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. Avoid assay of Samples containing Sodium Azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Anti-Teriparatide.

3. It is recommended that all Standards and Samples be assayed in duplicates.
4. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
5. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
6. The plates should be read within 30 minutes after adding the Stop Solution.
7. Make a work list in order to identify the location of Standards and Samples.

Procedure

1. It is strongly recommended that all Controls and Samples be run in duplicates. A standard curve is required for each assay. All steps must be performed at 37°C.
2. Add **100 µl** of **Standards or Samples** into the respective wells.
3. Add **100 µl** of **Teriparatide: HRP Conjugate** into each well.
4. Cover the plate and incubate for 60 minutes at 37°C.
5. Aspirate and wash plate 5 times with **Wash Buffer (1×)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.
6. Add **100 µl** of **TMB Substrate** in each well.
7. Incubate the plate at Room Temperature for 30 minutes in dark. **DO NOT SHAKE** or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.
8. Pipette out **100 µl** of **Stop Solution**. Wells should turn from blue to yellow in color.
9. Read the absorbance at 450 nm with a microplate reader.

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

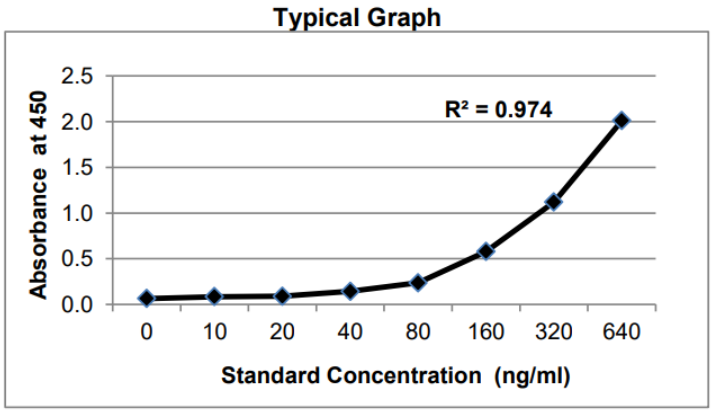
Determine the Mean Absorbance for each set of duplicate Standards and Samples. Using graph paper, plot the average value (absorbance 450 nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Anti-Teriparatide concentrations, find the unknown' s Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Anti-Teriparatide Concentration. If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit or a polynomial curve (2^{nd} order) is best recommended for automated results.

Note:

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

1. If the sample absorbance value is below the first standard.
2. If the absorbance value is equivalent or higher than the 640 ng/ml standard.

Typical Standard Curve



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 (10 ng/ml), medium (80 ng/ml) and high (640 ng/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	<12%	<12%
Medium	<10%	<10%
High	<10%	<10%

Sensitivity

Limit Of Detection: 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 10 ng/ml.

Specificity

The antibodies used in the kit are monoclonal antibodies, anti-idiotypic and specific for Anti-Teriparatide. The calibrators/standards used are calibrated against commercially sourced.

Linearity

Standards provided in the kit will be used for measuring the linearity range of Anti-Teriparatide 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 - Do not smoke, eat or drink while handling kit material - Always use protective gloves - Never pipette material by mouth - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
10. In any case GLP should be applied with all general and individual regulations to the use of this kit.