Anti-PTH Antibody ELISA

REF: KBI8000

Ver 1.0

RUO

Immunoassay for the quantitative determination of Anti-PTH Antibody in serum and plasma

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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KRISHGEN BioSystems Unit Nos#318/319, Shah & Nahar, Off Dr E Moses Road, Worli, Mumbai 400018.
Tel: 022-49198700 | Email:sales@krishgen.com

Introduction:

Teriparatide (rhPTH 1-34) is a recombinant human parathyroid hormone derivative consisting of the first 34 amino acids of the hormone. Teriparatide was approved as a drug by the FDA and is sold by Eli Lilly & Co. as Forteo. Teriparatide is indicated for use in patients with osteoporosis. Immunogenicity for this drug, was detected in 2.8% of women receiving Teriparatide during clinical trials of Forteo. The detection of these circulating antibodies may be of clinical relevance for the proper assessment of patients.

Intended Use:

This Anti-PTH Antibody ELISA is a rapid and easy method for the qualitative determination of Anti-PTH Antibody in human serum and plasma.

Principle:

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

Materials Provided:

1. Human PTH (1-34) coated Microtiter plate (12x8 wells) – 1 no
2. Anti-PTH Antibody Controls (Three Level Controls; 1, 2, 3) – 3 vials
3. Human PTH (1-34):HRP Conjugate, 12ml – 1 bottle.
4. Wash Buffer (20X), 50 ml – 1 bottle.
5. Sample Diluent (20X), 50 ml – 1 bottle.
6. TMB Substrate, 12 ml – 1 bottle.
7. Stop Solution, 12 ml – 1 bottle.
8. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettes to measure volumes ranging from 25 ul to 1000 ul
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

Handling/Storage:

1. All reagents should be stored at 2ºC to 8ºC for stability.
2. All the reagents and wash solutions should be used within expiry date as mentioned on kit.
3. Before using, bring all components to room temperature (18-25ºC). 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.
5. Lyophilized standard should be stored at 2ºC to 8ºC. Reconstituted standard can be kept at 4ºC to 8ºC upto 48 hours. Do not freeze.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research or Manufacturing use only.

Sample Preparation and 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 -80ºC. 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.

Note: Patient samples have to be diluted with 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.

Reagent Preparation (all reagents should be diluted immediately prior to use):

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.
4. To make Sample Diluent (1X) : Dilute 5 ml of (20X) Sample Diluent in 95 ml of DI water. This is the working solution.
5. Reconstitute Anti-PTH Antibody Control vials in 500 ul of sterile water. Allow to stand for $\geq$ 10 minutes to dissolve completely, prior to making dilutions.

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Anti-PTH Antibody. High Dose Hook Effect is due to excess of antibody for very high concentrations of Anti-PTH Antibody present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent. Thus if the Anti-PTH Antibody concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
3. 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-PTH Antibody.
4. It is recommended that all Standards and Samples be assayed in duplicates.

5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.

6. 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.

7. The plates should be read within 30 minutes after adding the Stop Solution.

8. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

Bring all reagents to room temperature prior to use. 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 room temperature (RT).

1. Pipet 50 ul of **Controls** or **Samples** into respective wells.

2. Pipet 50 ul of **Assay Buffer** into each well.

3. **Incubate** at Room Temperature for **60 minutes**.

5. Aspirate and wash plate 4 times with **300 ul Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

6. Pipet **100 ul** of **Human PTH (1-34):HRP Conjugate** into each well.

7. **Incubate** plate at Room Temperature for **60 minutes**.

9. Aspirate and wash plate 4 times with **300 ul Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

10. Pipet 100 ul of TMB Substrate into each well.

11. **Incubate** at Room Temperature for **30 minutes** under dark.

12. Pipet **50 ul** of **Stop Solution** into each of the wells. Mix gently.

14. Read the absorbance at 450 nm within 10 minutes in a microtiter plate reader.

Calculation of Results:

Subtract Blank absorbance value from all wells.
*Cut Off Value = Average of NC / 2 * 0.3*

Negative Control (NC): [NC1+NC2] / 2

Cut Off Value: Mean of NC+0.3

Validation Criteria:
Positive Control Value > 1.0
Negative Control Value < 0.3

Interpretation of Results:
Positive Sample Value = OD > Cut Off Value
Negative Sample Value = OD < Cut Off Value

Safety Precautions:

Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- 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.
- 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.
- 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.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

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.

A Typical Assay Setup

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	NC	S4	S8	S12	S16	S20	S24	S30	S34	S38	S42
B	PC1	S1	S5	S9	S13	S17	S21	S25	S31	S35	S39	S43
C	PC1	S1	S5	S9	S13	S17	S21	S26	S31	S35	S39	S43
	PC2	S2	S6	S10	S14	S18	S22	S27	S32	S36	S40	S44
E	PC2	S2	S6	S10	S14	S18	S22	S28	S32	S36	S40	S44
F	PC3	S3	S7	S11	S15	S19	S23	S29	S33	S37	S41	S45
G	PC3	S3	S7	S11	S15	S19	S23	S29	S33	S37	S41	S45
H	NC	S4	S8	S12	S16	S20	S24	S30	S34	S38	S42	Blank

* All controls and samples are run in duplicates

Blank **–** Blank wells
P.C **–** Positive control wells
N.C **–** Negative control wells
S. **–** Sample extract wells

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