ab325032 – Teriparatide (PTH -34) ELISA Kit

For the quantitative determination of Teriparatide (PTH -34) in human plasma and cell culture supernatant.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab325032

Storage and Stability: Store the whole kit at 2-8°C immediately upon receipt. Please refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent preparation sections.

Materials Supplied

Item	Quantity 1 x 96 tests	Storage
Streptavidin coated Microtiter plate (12x8 wells)	1 unit	2-8°C
Biotinylated Anti-PTH Antibody (concentrated)	2.7 mL	2-8°C
Standards	7 vials	2-8°C
Anti-PTH:HRP Conjugate (concentrated)	2.7 mL	2-8°C
Control 1	1 vial	2-8°C
Control 2	1 vial	2-8°C
(20X) Wash Buffer	20 mL	2-8°C
TMB Substrate	22 mL	2-8°C
Stop Solution	12 mL	2-8°C

Materials Required, Not Supplied

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

- Microplate Reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 50 µL to 100 µL
- Deionized (DI) water
- Wash bottle or automated microplate washer
- Horizontal rotator capable of maintaining 180 - 220 rpm
- Container for storage of wash solution
- Spectrophotometric microtiter plate reader capable of reading absorbance at 450 nm and at 595-650 nm
- Timer

Storage Information

- All reagents should be stored at 2°C to 8°C. Store Standards and Controls at -20°C or below after reconstitution.
- All the reagents and wash solutions are stable until the expiration date of the kit.
- 30 minutes prior before use, bring all components to room temperature (18-25°C). Store all the components of the kit at its appropriate storage condition after use.
- The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Specimen Collection and Handling

- Specimens should be clear and non-hemolyzed.
- Samples should be run at a number of dilutions to ensure accurate quantitation.
- Measurement of the PTH-34 concentration should be made using EDTA plasma or cell culture media.
- Three hundred microliters of plasma or media are required to assay the sample in duplicate.
- Centrifuge the sample and separate the plasma or media from the cells.
- Samples should be assayed immediately or stored frozen at -20°C or below. Avoid repeated freezing and thawing of specimens.

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

- **Control 1 and Control 2:** Reconstitute the controls in 1 mL of DI water. Allow the vials to sit for approximately 20 minutes with occasional gentle swirling and inversion. Assure complete reconstitution before use. Use the controls immediately after reconstitution.
- **Standards:** Standard\ 0 pg/mL: Reconstitute the vial in 2.0 mL of DI water.
- **Other Standards:** Reconstitute the vials in 1.0 mL of DI water.
- **(1X) Wash Buffer:** Add 20 mL (20X) Wash Buffer in 380 mL of deionized water.
- **Working Antibody Solution:** Make a Working Antibody Solution by mixing equal volumes of Biotinylated Anti-PTH Antibody (concentrated) and Anti-PTH:HRP Conjugate (concentrated) before use. Mix only the volume required for immediate use. Mix well to ensure homogeneity.

Procedural Notes

- In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
- Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Liraglutide.
- It is recommended that the Standards and Samples be assayed in duplicates.
- Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
- If the Substrate has a distinct blue color prior to use it may have been contaminated, and use of such substrate can lead to the sensitivity of the assay being compromised.
- The plates should be read within 30 minutes after adding the Stop Solution.
- Make a work list in order to identify the location of Standards and Samples.

Assay Procedure

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all Standards, Controls and Samples should be run in duplicates. A standard curve is required for each assay.
2. Pipette 150 µL of Standards, Controls, or samples into the designated or mapped well. Freeze the remaining standards as soon as possible after use.
3. Pipette out 50 µL of Working Antibody Solution consisting of 1 part Biotinylated Anti-PTH Antibody and 1 part Anti-PTH:HRP Conjugate into each well.
4. Cover the plate and incubate for 3 hours at RT on shaker condition (at 180-220 rpm).

5. Aspirate and wash plate 5 times with 350 µL of (1X) Wash Buffer 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. All the washes should be performed similarly.
6. Add 200 µL of TMB Substrate to each well and cover the plate.
7. Incubate for 30 minutes at RT in dark on shaker condition (at 180-220 rpm).
8. Pipette 50 µL of Stop Solution into each of the wells.
9. Read the absorbance at 450 nm within 10 minutes in a microtiter plate reader.

Calculation of Results

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using graph paper, plot the net 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 Teriparatide (PTH-34) 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 Teriparatide (PTH-34) Concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a cubic spline curve-fit, 4PL or a polynomial curve (2nd order) is best recommended for automated results.

∆***Note:*** *It is recommended to repeat the assay at a different dilution factor if the sample absorbance value is below the first standard or if the absorbance value is equivalent or higher than the highest standard.*

Limitations of the Procedure

- The reagents in this kit have been optimized so that the high dose "hook effect" is not a problem for samples with elevated PTH-34 values. Samples with levels between the highest standard and 800,000 pg/mL will read greater than the highest standard and should be diluted 1:10 or more with the 0 pg/mL Standard and re-assayed for correct values.
- Grossly lipemic plasma samples may affect the immunological response and it is recommended that results obtained with such samples be scrutinized accordingly.
- Differences in protein concentration and protein type between samples and standards in an immunoassay contribute to "protein effects" and dose biases. When measuring low protein concentration culture media samples against high protein concentration standards, it is recommended that like samples be assayed together in the same assay to minimize this bias.

Additional information

Precision

To assess Intra-assay precision the mean and coefficient of variation were calculated from 20 duplicate determinations of two samples each performed in a single assay.

Mean Value (pg/mL)	Coefficient of Variation
22.3	2.6%
127	2.1%

To assess Inter-assay precision the mean and coefficient of variation were calculated from duplicate determination s of two samples performed in 11 assays.

Mean Value (pg/mL)	Coefficient of Variation
23.1	8.5%
130	6.2%

Cross Reactivity

The following cross-reactants were diluted in the 0 pg/mL Standard and measured using the kit. The results are expressed as % cross-reactivity relative to the human PTH-34 standards contained in the kit.

Cross-Reactant Measured	Cross-Reactivity	
	Weight Basis	Molar Basis
Human PTH-34	100.0	100.0
Human PTH (1-84)	6.5	2.8
Human PTH (7-84)	<0.001	<0.0004
Rat PTH-34	43.0	43.6
Rat PTH (1-84)	6.8	3.0

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide
For technical support contact information, visit: www.abcam.com/contactus

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