Epitope Diagnostics, Inc.

FOR REFERENCE USE ONLY

EDI™ Human Intact PTH ELISA

Enzyme Linked ImmunoSorbent Assay (ELISA) for the Quantitative Measurement of Human Intact Parathyroid hormone (PTH) Levels in EDTA-Plasma

REF KT-888 EU: IVD CE 96 +2 +8 °C

I. INTENDED USE

This test kit is intended for use in the quantitative determination of human intact parathyroid hormone (PTH) in EDTA-plasma. The test is useful for detecting elevated and deficient PTH levels.

Indications for use:

Patient may have a higher than normal levels of PTH with

1. Hypercalcemia, Hypophosphatemia
2. Primary Hyperparathyroidism (adenoma, hyperplasia, malignancy)
3. Secondary Hyperparathyroidism (Vitamin D deficiency, kidney insufficiency, malabsorption-syndrome, pseudohyperparathyroidism)

Patient may have a lower than normal levels of PTH with

4. High doses of vitamin-D
5. Hypercalcemia of malignancy
6. Milk-alkali-syndrome
7. Morbus Boeck (Sarcoidosis)
8. Hyperthyreosis

II. SUMMARY OF PHYSIOLOGY

Parathyroid hormone PTH is a 84 amino acid polypeptide with an approximate molecular weight of 9500 Dalton. PTH is the most important endocrine regulator of calcium and phosphorus concentration in extracellular fluid. This hormone is secreted from cells of the parathyroid glands and finds its major target cells in bone and kidney.

III. ASSAY PRINCIPLE

This ELISA kit is designed, developed and produced for the quantitative measurement of human PTH in EDTA-plasma sample. The assay utilizes the two-site “sandwich” technique with selected antibodies that bind to N-terminal and mid-region epitopes of PTH.

Assay standards, controls and patient samples are added directly to wells of a microtiter plate that is coated with antibody to the N-terminal of human PTH. After the first incubation period, unbound material in the sample is removed in subsequent washing step. A horseradish peroxidase (HRP) conjugated anti mid-region of human PTH antibody is added to each well. After the second incubation period, a “sandwich” of solid-phase polyclonal antibody - human PTH – HRP conjugated monoclonal antibody” is formed. The unbound antibodies and buffer matrix are removed in the subsequent washing step. For the detection of this immunocomplex, the well is then incubated with a substrate solution in a timed reaction, which is terminated with an acidic reagent (i.e. ELISA stop solution). The absorbance is then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to the wall of each microtiter well is directly proportional to the amount of human PTH in the test sample. A standard curve is generated by plotting the absorbance versus the respective human PTH concentration for each standard on a point-to-point or 4-parameter curve fitting. The concentration of human PTH in test samples is determined directly from this standard curve.

IV. REAGENTS: Preparation and Storage

This test kit must be stored at 2 – 8 °C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Prior to use allow all reagents to come to room temperature. Regents from different kit lot numbers should not be combined or interchanged.

1. Anti-PTH Antibody Coated Microplate (Cat. No. 31030)
One microplate with twelve by eight strips (96 wells total) coated with polyclonal anti-human PTH N-terminus antibody. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

2. Anti-PTH Tracer Antibody (Cat. No. 31031)
One vial containing 1.2 mL HRP-labeled anti-human PTH mid-regional monoclonal antibody in a stabilized protein matrix. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

3. ELISA Wash Concentrate (Cat. No. 10010)
One bottle containing 30 mL of 30-fold concentrate. Before use the contents must be diluted with **870 mL** of demineralized water and mixed well. Upon dilution, this yields a working wash solution containing a surfactant in phosphate-buffered saline with a non-azide, non-mercury preservative. The diluted wash solution may be stored at room temperature and is stable until the expiration date on the kit box.

4. ELISA HRP Substrate (Cat. No. 10020)
One bottle containing 24 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

5. ELISA Stop Solution (Cat. No. 30357)
One bottle containing 15 mL of stop solution. This reagent may be stored at 2 – 8 °C or room temperature and is stable until the expiration date on the kit box.

6. Human PTH Standards (Cat. No. 31021-31026)
Six vials containing human PTH (1-84) in a lyophilized bovine serum-based matrix with a non-azide preservative. **Refer to the vials for exact concentration of the standard.** These standards should be stored at 2 – 8 °C and are stable until the expiration date on the kit box. Refer to assay procedure section for reconstitution directions.

7. Human PTH Controls (Cat. No. 31027 – 31028)
Two vials containing human PTH (1-84) in a lyophilized bovine serum-based matrix with a non-azide preservative. **Refer to vials for exact concentration range for each control.** Both controls should be stored at 2 – 8 °C and are stable until the expiration date on the kit box. Refer to assay procedure section for reconstitution instructions.

8. Tracer Antibody Diluent (Cat. No. 31032)
One bottle containing 24 mL each of ready-to-use buffer. It should be used only for tracer antibody dilution according to the assay procedures. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

V. SAFETY PRECAUTIONS

The reagents must be used in professional laboratory. Source material for reagents containing bovine serum was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

VI. MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 25 µL, 200 µL, etc.
2. Disposable pipette tips suitable for above volume dispensing.
3. Aluminum foil.
4. Deionized or distilled water.
5. Plastic microtiter well cover or polyethylene film.
6. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
7. Spectrophotometric microplate reader capable of reading absorbance at 450/650 or 450/620 nm.

VII. SPECIMEN COLLECTION

EDTA-plasma is a suitable specimen for human PTH measurement. A total of 0.4 mL EDTA-plasma is required for duplicate determination of human PTH with this test kit. Whole blood should be collected using lavender-top Vaccutainer and the plasma separated according to manufacturer's instruction. The EDTA-plasma should be separated from other cells right after or within one hour of blood collection. The plasma should be transferred to a clean test tube right after centrifugation. **Plasma samples should be stored at – 20ºC** if the assay is not to be performed within 3 hours. Avoid more than three times freeze-thaw cycles of specimen.

Samples of serum, heparin plasma and citrate plasma should not be used for PTH measurement.

VII. ASSAY PROCEDURE

1. Reagent Preparation

(1) Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

(2) ELISA Wash Concentrate (Cat. 10010) must be diluted to working solution prior to use. Please see REAGENTS section for details.

(3) Reconstitute assay standards and controls by adding **2.0 mL** of deminerialized water to each standard and control bottle. Allow the standard and controls to sit undisturbed for 5 minutes, and then mix well by inversions or **gentle** vortexing. One must make sure that all solid is dissolved completely prior to use.

(4) These reconstituted standards and controls may be stored at 2- 8°C for up to 24 hours or below –10 °C for long-term storage. Do not exceed 3 freeze-thaw cycles.

(5) Prepare Tracer Antibody working solution by 1:21 fold dilution of the PTH Tracer Antibody (Cat. 30639) by adding the tracer antibody into the Tracer Antibody Diluent (Cat. 31032). **Add 100µL of Tracer Antibody for every 2mL of Tracer Antibody Diluent** . **NOTE:** the tracer antibody should be prepared just prior to the first washing step. If 1 whole plate is used, just transfer all the Tracer Antibody into the Tracer Antibody Diluent bottle.

(6) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3	STRIP 4
A	STD 1	STD 5	SAMPLE 1	SAMPLE 5
B	STD 1	STD 5	SAMPLE 1	SAMPLE 5
C	STD 2	STD 6	SAMPLE 2	SAMPLE 6
D	STD 2	STD 6	SAMPLE 2	SAMPLE 6
E	STD 3	C 1	SAMPLE 3	
F	STD 3	C 1	SAMPLE 3	
G	STD 4	C 2	SAMPLE 4	
H	STD 4	C 2	SAMPLE 4	

(7) Place a sufficient number of Anti-PTH antibody-coated microwell strips in a holder to run human PTH standards, controls and unknown samples in duplicate.

2. Assay Procedure:

(1) Add **200 µL** of standards, controls and patient samples into the designated microwells.

(2) Seal the plate wells securely, cover with foil or other material to protect from light, and rotate on an ELISA plate shaker (small orbit radius) for 2 hr. ± 5 minutes at 400 to 450 rpm.

(3) Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.

(4) Immediately add **200 µL** of HRP Conjugated Anti-PTH Tracer Antibody mix to each well.

(5) Seal the plate wells securely, cover with foil or other material to protect from light, and rotate on an ELISA plate shaker (small orbit radius) for 1 hr. ± 5 minutes at 400 to 450 rpm

(6) Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used

(7) Add **200 µL** of ELISA HRP Substrate (Cat. 10020) into each of the wells.

(8) Cover the plate with aluminum foil or other material to avoid exposure to light. Incubate plate static, at room temperature for **20 minutes**.

(9) Immediately add **50 µL** of ELISA Stop Solution (Cat. 30557) into each of the wells. Mix gently.

(10) Read the absorbance at 450 nm with reference filter at 620 nm or 650 nm.

VIII. PROCEDURAL NOTES

1. It is recommended that all standards, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original amber bottles.
3. Store any unused antibody-coated strips in the foil zipper bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. An orbital mixer with a larger orbit radius (e.g. > 1 cm) may be used at speeds of 150 to 200 rpm.

7. Avoid air bubbles in the microwells as this could result in lower binding efficiency and higher CV% of duplicate reading.
8. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.
9. If adapting this assay to automated ELISA system such as DS-2 (Dinex Corp., Miami), a procedural validation is necessary if there is any modification of the assay procedure.

IX. INTERPRETATION OF RESULTS

It is recommended to use a point-to-point standard curve fitting.

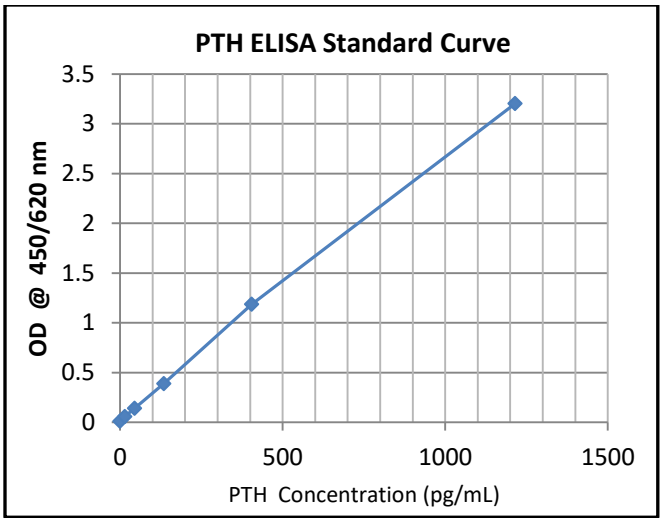
1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the level 1 standard (0 pg/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The standard curve is generated by the corrected absorbance of all standard levels on the ordinate against the standard concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results.

The human PTH concentrations for the controls and the patient samples are read directly from the standard curve using their respective corrected absorbance.

X. EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting standard curve from this EDTA plasma PTH ELISA are represented. **This curve should not be used in lieu of standard curve generated with each assay.**

Well I.D.	Absorbance 450/620 nm			Results
	Readings	Average	Corrected	
Std-1: 0 pg/mL	0.011 0.011	0.011	0.000	
Std-2: 15 pg/mL	0.057 0.058	0.058	0.047	
Std-3: 45 pg/mL	0.143 0.139	0.141	0.130	
Std-4: 135 pg/mL	0.387 0.392	0.389	0.378	
Std-5: 405 pg/mL	1.188 1.187	1.187	1.176	
Std-6: 1215 pg/mL	3.200 3.207	3.204	3.193	
Control 1	0.109 0.111	0.110	0.099	33.8 pg/mL
Control 2	0.779 0.773	0.776	0.765	265.7 pg/mL



XI. EXPECTED VALUES

Eighty EDTA plasma samples from normal healthy adults ages 21 – 64 were collected and measured with this ELISA.
Results for PTH concentration by using this ELISA were from 7.5 - 63 pg/mL. We strongly recommend for each clinical laboratory to establish its own normal range by measuring EDTA plasma samples with this ELISA. Please note that sample collection time may have impact on the PTH normal range.

XII. LIMITATION OF THE PROCEDURE

1. This PTH assay requires EDTA-plasma sample for testing. Serum sample may show a lower PTH level and must not be used, because PTH is not stable in serum.
2. For sample values reading greater than the highest standard, it is recommended to re-assay samples with dilution (i.e. 1:10 or 1:100 with standard zero).
3. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.

XIII. QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls.

XIV. PERFORMANCE CHARACTERISTICS

The analytical sensitivity (LLOD) of the PTH ELISA as determined by the 95% confidence limit on 16 replicate determinations of zero standard is less than 1 pg/mL (0.77 pg/mL).

High Dose "hook" effect
The PTH Standard Matrix was spiked with increasing concentrations of PTH 1-84. This assay has showed no high dose "hook" for PTH levels up to 25,000 pg/mL.

Precision
The intra-assay precision was validated by measuring two control samples with 16 replicate determinations.

Sample #	Mean PTH Value (pg/mL)	CV (%)
1	30.5	2.0
2	307.9	1.9

The inter-assay precision was validated by measuring two control levels in duplicate in 12 individual assays.

Sample #	Mean PTH Value (pg/mL)	CV (%)
1	32.1	9.5
2	266.1	5.6

Linearity
Two PTH standard levels were diluted with standard zero (i.e. level 1 standard) and tested. The results of PTH percent recovery value in pg/mL are as follows:

DILUTION	OBSERVED VALUE (pg/mL)	EXPECTED VALUE (pg/mL)	RECOVERY %
Neat A	254.8	-	-
1:2	111.5	127.4	87
1:4	58.7	63.7	92
1:8	31.6	31.8	99
Neat B	405	-	-
1:2	183.8	202.5	91
1:4	86.7	101.3	86
1:8	49.0	50.6	97

Spike Recovery
Two PTH Standard levels and three assay standards (45, 135 and 405 pg/mL) were combined at equal volumes and tested. The results are as follows:

DILUTION	OBSERVED VALUE (pg/mL)	EXPECTED VALUE (pg/mL)	RECOVERY %
Neat A	33.4	-	-
Std-3	39.3	39.2	100
Std-4	76.5	84.2	91
Std-5	206.3	219.2	94
Neat A	266.2	-	-
Std-3	152.6	155.6	98
Std-4	185.9	200.6	93
Std-5	322.2	335.6	96

Cross-Reactivity
100% of High concentrations of the following peptides/protein were measured using this ELISA.

Cross-reactant	Concentration *(ng/ml)*	% Cross-Reactivity
ACTH (1-39)	10	< 0.00000
Insulin	20	<0.000313
Fetuin - A	270	<0.000006
C- peptide	50	<0.000008
25-OH-D2	1000	<0.000001
25-OH-D3	100	<0.000016
Osteocalcin	60.7	<0.000009

Interference
Interference was tested by spiking (95%) EDTA plasma samples with (5%) concentrations of hemoglobin, lipid, and bilirubin. The results are provided below:

Sample	Measured Value (pg/mL)	Interferant added (mg/mL)
Test Control	**23.5**	**buffer**
Billirubin	23.9	0.4
	22.5	2
	21.4	10
Test Control	**8.8**	**buffer**
Hemoglobin	9.5	0.4
	10.0	2
	9.9	10
Lipid	8.8	8
	8.9	40
	8.7	200

Sample	Measured Value (pg/mL)	Interferant added (mg/mL)
Test Control	**12.6**	**buffer**
Billirubin	13.2	0.4
	13.1	2
	12.1	10
Test Control	**22.5**	**buffer**
Hemoglobin	24.4	0.4
	25.5	2
	30.3	10
Lipid	24.7	8
	23.9	40
	21.8	200

XV. WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

XVI. REFERENCES

1. National Institute of Health
2. Calcium Homeostasis in Health and in Kidney Disease
3. Moe, SM, Compr Physiol 2016 Sep 15;6(4):1781-1800. Epub 2016 Sep 15.
4. Alsahw, N, Blavo, D, Karanchi, H, Systemic sarcoidosis with hypercalcaemia, hypothalamic-pituitary dysfunction and thyroid involvement.**,** BMJ Case Rep 2016 Aug 5;2016. Epub 2016 Aug 5.
5. Keutmann, H.T., Sauer, M.M., Hendy, G.N., O'Riordan, J.L.H., and Potts Jr., J.T. "Complete Amino Acid Sequence of Human Parathyroid Hormone." Biochemistry 17:5723-5729, 1978
6. Habener, J.F., and Potts Jr., J.T. "Biosynthesis of Parathyroid Hormone." New England Journal of Medicine 299:580-585, and 635-644, 1978.
7. Dambacher, M.A., Fischer, J.A., Hunziker, W.H. et. al. "Distribution of Hormone in Normal Subjects and in Patients Circulating Immunoreactive Components of Parathyroid with Primary and Secondary Hyperparathyroidism: The Role of the Kidney and of the Serum Calcium Concentration." Clinical Science 57:435, 1979.

TECHNICAL ASSISTANCE AND CUSTOMER SERVICE
For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.
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Manufacturer	No. of tests
Catalog Number	Keep away from heat and direct sun light
Concentrate	Store at
In Vitro Diagnostic Device	Use by
Read instructions before use	Lot No.
Authorized Representative In Europe	

PTH ELISA: Condensed Assay Protocol

1. 200 µL standards, controls and patient samples

↓ *Incubate @ RT for 2 hours on ELISA plate shaker Wash 5 x*

2. 200 µL Tracer Antibody

↓ *Incubate @ RT for 1 hour on ELISA plate shaker Wash 5 x*

3. 200 µL TMB Substrate

↓ *Incubate @ RT for 20 min static*

4. 50 µL Stop Solution *Immediately*

↓

5. Read absorbance at 450/650 or 450/620 nm *within 10 minutes*