Human Intact PTH ELISA Assay Kit

Catalog Number:
PTH31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0 (03.23.20)

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INTENDED USE

This Eagle Biosciences Human Intact PTH ELISA Assay 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. The Eagle Biosciences Human Intact PTH ELISA Assay Kit is for research use only and not used for diagnostic procedures.

BACKGROUND

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.

PRINCIPLE OF THE ASSAY

This Eagle Biosciences Human Intact PTH ELISA Assay 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 calibrators, 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 calibration curve is generated by plotting the absorbance versus the respective human PTH concentration for each calibrator on a point-to-point or 4-parameter curve fitting. The concentration of human PTH in test samples is determined directly from this calibration curve.

REAGENTS: Preparation and Storage
The Eagle Biosciences Human Intact PTH ELISA Assay 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
Coated with polyclonal anti-human PTH N-terminus antibody.
Qty: 1 x 96 well microplate
Storage: 2 – 8°C
Preparation: Ready to Use.

2. Anti-PTH Tracer Antibody
HRP-labeled anti-human PTH mid-regional monoclonal antibody in a stabilized protein matrix.
Qty: 1 x 1.2 mL
Storage: 2 – 8°C
Preparation: Ready to Use.

3. ELISA Wash Concentrate
Surfactant in a phosphate buffered saline with non-azide preservative.
Qty: 1 x 30 mL
Storage: 2 – 25°C
Preparation: 30X Concentrate. The contents must be diluted with 870 mL distilled water and mixed well before use.

4. ELISA HRP Substrate
Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.
Qty: 1 x 24 mL
Storage: 2 – 8°C
Preparation: Ready to Use.

5. ELISA Stop Solution
1.0 M sulfuric acid
Qty: 1 x 12 mL
Storage: 2 – 25°C
Preparation: Ready to Use.

6. Human PTH Calibrators Levels 1 – 6
Human PTH (1-84) in a lyophilized bovine serum-based matrix with a non-azide preservative. Refer to the vials for exact concentration.
Qty: 6 x Vials
Storage: 2 – 8°C (Lyophilized), <-20°C (Reconstituted)
Do not exceed 3 freeze-thaw cycles.
Preparation: Must be reconstituted with 2.0 mL of demineralized water, allowed to sit for 10

minutes, and then mix microwell by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

7. Human PTH Controls

Human PTH (1-84) in a lyophilized bovine serum-based matrix with a non-azide preservative. Refer to the vials for exact concentration.

Qty: 2 x Vials
Storage: 2 – 8°C (Lyophilized), <-20°C (Reconstituted)
Do not exceed 3 freeze-thaw cycles.
Preparation: Must be reconstituted with 2.0 mL of demineralized water, allowed to sit for 10 minutes, and then mix microwell by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

8. Tracer Antibody Diluent

For tracer antibody dilution according to the assay procedures.

Qty: 1 x 24 mL
Storage: 2 – 8°C
Preparation: Ready to Use.

SAFETY PRECAUTIONS

The reagents are for research use only. Source material which contains reagents of bovine serum albumin 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 were potentially infectious. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid. 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.

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.

SPECIMEN COLLECTION & STORAGE

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.

ASSAY PROCEDURE

ASSAY PROCEDURE

1. **Reagent Preparation**
   a. Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
   b. ELISA Wash Concentrate must be diluted to working solution prior to use. Please see REAGENTS section for details.
   c. Reconstitute assay calibrators and controls by adding **2.0 mL** of deminerialized water to each calibrator and control bottle. Allow the calibrator 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. These reconstituted calibrators and controls may be stored at 2- 8°C for up to 24 hours or below –20 °C for long-term storage. Do not exceed 3 freeze-thaw cycles.

2. **Manual Assay Procedure**

1. Place a sufficient number of microwell strips in a holder to run calibrators, controls, and samples in duplicate.
2. Test Configuration

Row	Strip 1	Strip 2	Strip 3
A	Calibrator Level 1	Calibrator Level 5	SAMPLE 1
B	Calibrator Level 1	Calibrator Level 5	SAMPLE 1
C	Calibrator Level 2	Calibrator Level 6	SAMPLE 2
D	Calibrator Level 2	Calibrator Level 6	SAMPLE 2
E	Calibrator Level 3	Control 1	SAMPLE 3
F	Calibrator Level 3	Control 1	SAMPLE 3
G	Calibrator Level 4	Control 2	SAMPLE 4
H	Calibrator Level 4	Control 2	SAMPLE 4

3. Add 200 µL of calibrators, controls, and samples into the designated microwells.
4. Cover the plate with one plate sealer and aluminum foil. Incubate at room temperature (20-25 ºC) with shaking at 400 to 450 rpm for 120 minutes.
5. Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of diluted wash solution into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
6. Prepare the Antibody Working Solution by 1:21 fold dilution of the tracer antibody with diluent. For each strip, it is required to mix 2 mL of the diluent with 100 µL of the antibody in a clean test tube.

Note: This antibody working solution should be freshly prepared.

7. Add 200 µL of the antibody working solution to each well.
8. Cover the plate with one plate sealer and aluminum foil. Incubate at room temperature (20-25 ºC) with shaking at 400 to 450 rpm for 60 minutes.

9. Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of diluted wash solution into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
10. Add 200 µL of the ELISA HRP substrate into each of the wells.
11. Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at room temperature (20-25 ºC) for 20 minutes.
12. Add 50 µL of the ELISA stop solution into each of the wells and mix gently.
13. Read the absorbance at 450/620 or 450/650 nm within 10 minutes with a microplate reader.

PROCEDURAL NOTES

1. It is recommended that all calibrators, 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.

INTERPRETATION OF RESULTS

1. It is recommended to use a point-to-point calibration curve fitting.
2. Calculate the average absorbance for each pair of duplicate test results.
3. Subtract the average absorbance of the level 1 calibrator. (0 pg/mL) from the average absorbance of all other readings to obtain corrected absorbance.
4. The calibration curve is generated by the corrected absorbance of all calibrator levels on the ordinate against the calibrator 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.
5. The human PTH concentrations for the controls and the patient samples are read directly from the calibration curve using their respective corrected absorbance.

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log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results.

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

LIMITATIONS OF THE PROCEDURE

1. This Eagle Biosciences 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 calibrator, it is recommended to re-assay samples with dilution (i.e. 1:10 or 1:100 with calibrator zero).
3. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.

QUALITY CONTROL

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

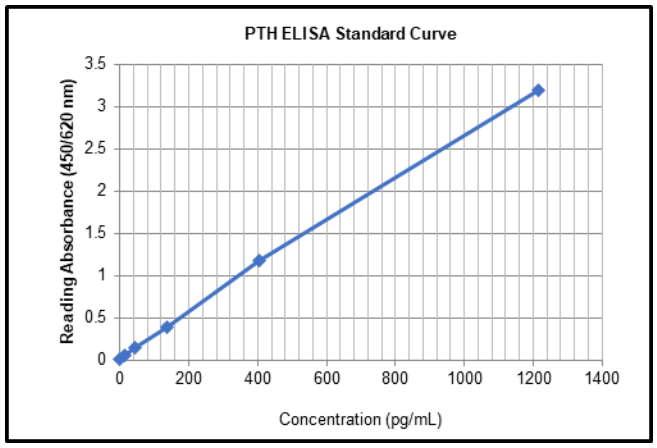
EXPECTED VALUES

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.

EXAMPLE DATA

A typical absorbance data and the resulting calibration curve from are represented.
Note: This curve should not be used in lieu of calibration curve run with each assay.

Well ID	Reading Absorbance (450/620 nm)			Concentration (pg/mL)
	Readings	Average	Corrected	
Calibrator Level 1: 0 pg/mL	0.011	0.011	0.000	
	0.011			
Calibrator Level 2: 15 pg/mL	0.057	0.058	0.047	
	0.058			
Calibrator Level 3: 45 pg/mL	0.143	0.141	0.130	
	0.139			
Calibrator Level 4: 135 pg/mL	0.87	0.389	0.378	
	0.392			
Calibrator Level 5: 405 pg/mL	1.188	1.187	1.176	
	1.187			
Calibrator Level 6: 1215 pg/mL	3.200	3.204	3.193	
	3.207			
Control 1	0.109	0.110	0.099	33.8
	0.111			
Control 2	0.779	0.776	0.765	265.7
	0.773			



PERFORMANCE CHARACTERISTICS

Sensitivity

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

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.

Reproducibility and Precision

The intra-assay precision was validated by measuring two control samples with 16 replicate determinations. The inter-assay precision was validated by measuring two control levels in duplicate in 12 individual assays. The results are as follows:

	Intra-Assay		Inter-Assay	
Sample	**1**	**2**	**1**	**2**
Mean (pg/mL)	30.5	307.9	32.1	266.1
CV (%)	2.0	1.9	9.5	5.6

Linearity

Two PTH calibrator levels were diluted with calibrator zero (i.e. level 1 calibrator) and tested. The results are as follows:

Samples	Observed (pg/mL)	Expected (pg/mL)	Recovery (%)
Sample A	254.8	-	-
50%	111.5	127.4	87
25%	58.7	63.7	92
12.5%	31.6	31.8	99
Sample B	405	-	-
50%	183.8	202.5	91
25%	86.7	101.3	86
12.5%	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 (pg/mL)	Expected (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. The results are as follows:

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 as follows:

Sample	Measured Value (pg/mL)	Interferant added (mg/mL)
Test Control	23.5	buffer
Bilirubin	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
Bilirubin	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

REFERENCES

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Warranty Information

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 617-419-2019.