ELISA PRODUCT INFORMATION & MANUAL

Parathyroid Hormone (PTH)

NBP2-60629

Enzyme-linked Immunosorbent Assay for quantitative detection of Human Parathyroid Hormone (PTH). For research use only.

Not for diagnostic or therapeutic procedures.

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Assay Summary

Step 1. Add 50 µl of Standard or Sample per well.
Incubate 2 hours.

Step 2. Wash, then add 50 µl of Biotinylated Antibody per well.
Incubate 1 hour.

Step 3. Wash, then add 50 µl of SP Conjugate per well.
Incubate 30 minutes.

Step 4. Wash, then add 50 µl of Chromogen Substrate per well.
Incubate 25 minutes.

Step 5. Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Assay Template

	A	B	C	D	E	F	G	H
1								
2								
3								
4								
5								
6								
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8								
9								
10								
11								
12								

Human Parathyroid Hormone (PTH) ELISA Kit

Catalog No. NBP2-60629

Sample insert for reference use only

Introduction

Parathyroid hormone (PTH), also known as parathormone and parathyrin, belongs to the parathyroid hormone family. It is biosynthesized as a 115-amino acid preproparathyroid hormone precursor. The prohormone is rapidly converted into the storage or glandular form of the hormone. PTH consists of 84 amino acids and has a molecular weight of 9500. Upon decreased serum calcium concentration stimuli, PTH is secreted into the circulation and is central to calcium homeostasis and bone maintenance (1-2). PTH binds to its G protein-coupled receptor and acts in an endocrine manner on kidney and bone cells to regulate blood levels of calcium, phosphate, and 1,25-dihydroxyvitamin D (3). In the kidney, PTH promotes renal tubular calcium resorption, stimulates the synthesis of 1,25-dihydroxyvitamin D, and inhibits reabsorption of phosphate. PTH increases osteoclastic bone resorption as part of calcium homeostasis. The stimulatory effects of PTH on osteoblasts increase bone mass (4).

Principle of the Assay

The AssayMax Human Parathyroid Hormone (PTH) ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human PTH in **plasma, serum, and cell culture samples**. This assay employs a quantitative **sandwich enzyme immunoassay** technique that measures human PTH in approximately 4 hours. A polyclonal antibody specific for human PTH has been pre-coated onto a 96-well microplate with removable strips. PTH in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for PTH, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This product is for **Research Use Only** and is not intended for use in diagnostic procedures.
- Prepare all reagents (diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.

- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
- Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.
- The Stop Solution is an acidic solution.
- The kit should not be used beyond the expiration date.

Reagents

- **Human Parathyroid Hormone Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human PTH.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human Parathyroid Hormone Standard:** Human PTH in a buffered protein base (1600 pg, lyophilized, 2 vials).
- **Biotinylated Human Parathyroid Hormone Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against PTH (120 µl).
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store Standard, SP Conjugate and Biotinylated Antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation, and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes and collect plasma. The sample is suggested for use at 1x; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes and remove serum. The sample is suggested for use at 1x; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes at 4°C to remove debris and collect supernatants. Samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 10-fold with reagent grade water. Store for up to 30 days at 2-8°C.
- **Human Parathyroid Hormone Standard:** Reconstitute the Human Parathyroid Hormone Standard (1600 pg) with 0.8 ml of MIX Diluent to generate a 2000 pg/ml standard stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (2000 pg/ml) 2-fold with MIX Diluent to produce 1000, 500, 250, 125, 62.5, and 31.25 pg/ml solutions. MIX Diluent serves as the zero standard (0 pg/ml). Any remaining stock solution should be frozen at -20°C and used within 2 days. **Aliquot standard to limit repeated freeze-thaw cycles.**

Standard Point	Dilution	[PTH] (pg/ml)
P1	1 part Standard (2000 pg/ml)	2000
P2	1 part P1 + 1 part MIX Diluent	1000
P3	1 part P2 + 1 part MIX Diluent	500
P4	1 part P3 + 1 part MIX Diluent	250
P5	1 part P4 + 1 part MIX Diluent	125
P6	1 part P5 + 1 part MIX Diluent	62.5
P7	1 part P6 + 1 part MIX Diluent	31.25
P8	MIX Diluent	0.0

- **Biotinylated Human Parathyroid Hormone Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 50-fold with MIX Diluent. The undiluted antibody should be stored at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 20-fold with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with MIX Diluent. The undiluted conjugate should be stored at -20°C.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Human Parathyroid Hormone Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 µl of Biotinylated Human Parathyroid Hormone Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour.
- Wash the microplate as described above.

- Add 50 μl of Streptavidin-Peroxidase Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 μl of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 25 minutes or until the optimal blue color density develops.
- Add 50 μl of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

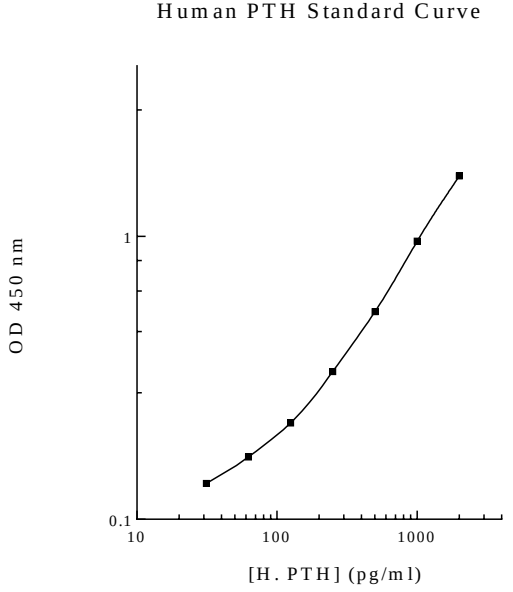
Typical Data

- The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	pg/ml	OD	Average OD
P1	2000	1.650 1.619	1.635
P2	1000	0.969 0.952	0.961
P3	500	0.546 0.538	0.542
P4	250	0.336 0.329	0.333
P5	125	0.225 0.213	0.219
P6	62.5	0.171 0.160	0.166
P7	31.25	0.136 0.131	0.134
P8	0.0	0.106 0.105	0.106
Sample: Pooled Normal Sodium Citrate Plasma (1x)		0.182 0.166	0.174

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of PTH as calculated by 2SD from the mean of a zero standard was established to be 29 pg/ml.
- Intra-assay precision was determined by testing three plasma samples twenty times in one assay.
- Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision			**Inter-Assay Precision**		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	3.2%	4.2%	3.9%	10.0%	10.2%	9.6%
Average CV (%)	3.8%			9.9%		

Recovery

Standard Added Value	62.5 – 1000 pg/ml
Recovery %	92 – 109%
Average Recovery %	96%

Linearity

- Plasma and serum samples were serially-diluted to test for linearity.

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
1x	98%	99%
2x	103%	102%
4x	105%	104%

Cross-Reactivity

Species	**Cross Reactivity (%)**
Canine	None
Bovine	None
Monkey	90%
Mouse	None
Rat	None
Swine	90%
Rabbit	None

Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of expired components	• Check the expiration date listed before use. • Do not interchange components from different lots.
	Improper wash step	• Check that the correct wash buffer is being used. • Check that all wells are empty after aspiration. • Check that the microplate washer is dispensing properly. • If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	• Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.
	Improperly sealed microplate	• Check the microplate pouch for proper sealing. • Check that the microplate pouch has no punctures. • Check that three desiccants are inside the microplate pouch prior to sealing.
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	• Each step of the procedure should be performed uninterrupted.
	Omission of step	• Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	• Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	• Check pipette calibration. • Check pipette for proper performance.
	Wash step was skipped	• Consult the provided procedure for all wash steps.
	Improper wash buffer	• Check that the correct wash buffer is being used.
	Improper reagent preparation	• Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	• Consult the provided procedure for correct incubation time.
Deficient Standard Curve Fit	Non-optimal sample dilution	• Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. • Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay. • User should determine the optimal dilution factor for samples.
	Contamination of reagents	• A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	• Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.

	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.

Version 1.2