Recovery

Various amounts of (PTH 25-100 pg/ml) were added to three different patient sera to determine the
recovery. Average sample recovery was 90-105%

Linearity of Patient Sample Dilutions: Parallelism

Four patient serum samples were diluted 1:2, 1:4, 1:8 with Reagent 4 (the Diluent for Patient
Samples read off-scale). Results in pg/mL are shown below

Sample A (initial conc 641/pg/ml) recovery 86-98%
Sample B (initial conc >700/pg/ml) recovery 93-109%
Sample C (initial conc 410/pg/ml) recovery 80-96%
Sample D (initial conc 649/pg/ml) recovery 81-98%

References: 1. Segre, G.V., Niall H.D., Habener J.F. et. al. : Metabolism of parathyroid hormone: physiological and clinical significance. Am. J. Med. 56: 774,1974; 2. Mallete, L.E., Gagel, R.F.: Parathyroid Hormone and Calcitonin. In: Murray J.F. (ed) Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. American Society for Bone and Mineral Research, Kelseyville; William Byrd Press, Richmond, pp. 65-69, 1990.

Related Items

Catalog#	Description
0010	Human Leptin ELISA Kit, 96 tests, Quantitative, 96 tests
0020	Human Beta-2 microglobulin (B2M) ELISA Kit, 96 tests, Quantitative
0030-10-B1	Bovine Insulin ELISA Kit, 96 tests, Quantitative, 96 tests
0030-20-I	Human Insulin-Biotin ELISA Kit, 96 tests, Quantitative, 96 tests
0030-40-1	Mouse Insulin ELISA Kit, High Sensitivity, Quantitative, 96 tests
0030-40-10	Mouse Insulin ELISA Kit, High Sensitivity, Quantitative, 10x 96 tests
0030-50-1	Rat Insulin ELISA Kit, High Sensitivity, Quantitative, 96 tests
0030-50-10	Rat Insulin ELISA Kit, High Sensitivity, Quantitative, 10x 96 tests
0030-60-1	Mouse/Rat Proinsulin ELISA Kit, High Sensitivity, Quantitative, 96 tests
0030-70-1	Mouse/Rat C-Peptide ELISA Kit, High Sensitivity, Quantitative, 96 tests
0030N	Human Insulin ELISA Kit, 96 tests, Quantitative, 96 tests
0035-IA	Human Insulin & Insulin Analogs (Lispro/Humalog, Aspart, Glargine, Glulisine, Detemir) ELISA Kit,
0040	Human C-peptide ELISA Kit, 96 tests, Quantitative
0050	Human Neuron Specific Enolase (NSE) ELISA Kit, 96 tests, Quantitative
0060	Human Pancreatic Colorectal cancer (CA-242/CA242) ELISA Kit, 96 tests, Quantitative
0070	Human Erythropoietin (EPO) Protein ELISA Kit, 96 tests, Quantitative
0080	Human Granulocyte Colony Stimulating Factor (G-CSF) Protein ELISA Kit, 96 tests, Quantitative
0100	Human Luteinizing Hormone (LH) ELISA Kit, 96 tests, Quantitative
0200	Human Folicle Stimulating Hormone (FSH) ELISA Kit, 96 tests, Quantitative
0300	Human Prolactin (PRL) ELISA Kit, 96 tests, Quantitative
0310	Human Parathyroid Hormone-Biotin (PTH-Biotin) ELISA Kit, 96 tests, Quantitative
0320-PTH	Human Parathyroid Hormone (PTH) ELISA Kit, 96 tests, Quantitative
0330-DDM	D-Dimer turbidimetric ELISA Kit, 96 tests, Quantitative
0340-B12	Vitamin B12 ELISA kit, 96 tests, Quantitative
0345-VID	Vitamin D total (25OH) ELISA kit, 96 tests, Quantitative
0350	Glucagon Like Peptide 1, Active (GLP-1, 7-36/7-37) ELISA kit, 96 tests, Quantitative
0360-0B6	Vitamin B6 ELISA kit, 96 tests, Quantitative
0365-0B9	Vitamin B9/Folic Acid (FA) ELISA kit, 96 tests, Quantitative
0370-HCY	Homocysteine ELISA kit, 96 tests, Quantitative
0380-PTC	Human Procalcitonin (PCT) ELISA Kit, 96 tests, Quantitative
0390-CTU	Human Calcitonin ELISA Kit, ultrasensitive, 96 tests, Quantitative
0400	Human Chorionic Gonadotropin (HCG) ELISA Kit, 96 tests, Quantitative

Instruction Manual No. M-0320-PTH

Human Parathyroid Hormone (PTH) ELISA KIT

Cat. # 0320-PTH, 96 tests

For Detecting Human Parathyroid Hormone (PTH) in Human Serum, plasma or other biological fluids

For In Vitro Research Use Only

ALPHA DIAGNOSTIC
INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.
Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777

Email: service@4adi.com
Web Site: www.4adi.com

Draft version-Please consult the manual supplied with the kit for any lot specific change.

Parathyroid Hormone ELISA KIT Cat. # 0320-PTH (96 tests)

Kit Components (96 tests)	Cat #
Streptavidin coated strip plate, (8x12 strip or 96 wells) # 0320-1P	1 plate
PTH **Standard A** (4mL) # 0320-2A	1 vial
PTH **Standard B Lyophilized** (0.5 mL) # 0320-2B	1 vial
PTH **Standard C Lyophilized** (0.5 mL) # 0320-2C	1 vial
PTH **Standard D Lyophilized** (0.5 mL) # 0320-2D	1 vial
PTH **Standard E Lyophilized** (0.5 mL) # 0320-2E	1 vial
PTH **Standard F Lyophilized** (0.5 mL) # 0320-2F	1 vial
PTH Control1 & Control 2, **Lyophilized** # 0320-2C1-C2 Lyophilized synthetic h-PTH. Lyophilized Ze ro calibrator [BSA solution with goat serum]. All other calibrators consist of synthetic h-PTH (1-84) in BSA	2 vials
Biotinylated PTH Antibody (7 ml) # 0320-3	1 bottle
Peroxidase (Enzyme) labeled PTH Antibody; 7 ml # 0320-4	1 bottle
TMB Substrate Solution, 20 ml # 0320-TMB	1 bottle
Diluent [equine serum] for Patient Samples read off-scale, 2 ml #0320-5	1 vial
Wash buffer conc. 30 ml # 0320-WB	1 bottle
Stop Solution, 20 ml # 0320-ST	1 bottle
Reconstitution Solution, 5 ml; #0320-6	1 bottle
Complete Instruction Manual, M-0320-PTH	1

Intended Use:

Intact-PTH ELISA is intended for the quantitative determination of Intact-PTH (Parathyroid Hormone) in human serum or plasma or other qualified samples. For research use only (RUO), not for diagnosis, cure or prevention of the disease.

Introduction:

PTH (Parathyroid hormone, Parathormone, Parathyrin) is biosynthesized in the parathyroid gland as a pre-proparathyroid hormone, a larger molecular precursor consisting of 115 amino acids. Following sequential intracellular cleavage of a 25-amino acid sequence, preproparathyroid hormone is converted to an intermediate, a 90-amino acid polypetide, proparathyroid hormone. With additional proteolytic modification, proparathyroid hormone is then converted to parathyroid hormone, an 84 amino acid polypeptide. In healthy individuals, regulation of parathyroid hormone secretion normally occurs via a negative feedback action of serum calcium on the parathyroid glands. Intact PTH is biologically active and clears very rapidly from the circulation with a half-life of less than four minutes. PTH undergoes proteolysis in the parathyroid glands, but mostly peripherally, particularly in the liver but also in the kidneys and bone, to give N-terminal fragments and longer lived C-terminal and midregion fragments. In subjects with renal insufficiency, C-terminal and midregion PTH assays typically give elevated PTH results, as reflected by impaired renal clearance.

Intact PTH assays are important for the differentiation of primary hyperparathyroidism from other (non-parathyroid-mediated) forms of hypercalcemia, such as malignancy, sarcoidosis and thyrotoxicosis2. The measurement of parathyroid hormone is the most specific way of making the diagnosis of primary hyperparathyroidism. In the presence of hypercalcemia, an elevated level of parathyroid hormone virtually establishes the diagnosis. In over 90% of patients with primary hyperparathyroidism, the parathyroid hormone will be elevated. Unlike C-terminal and midregion PTH, which typically are grossly elevated in subjects with renal insufficiency, intact PTH assays are less influenced by the declining renal function

Quality Control

Control serum or serum pools should be analyzed with each run of calibrators and patient samples. Results generated from the analysis of the control samples should be evaluated for acceptability using appropriate statistical methods. In assays in which one or more of the quality control sample values lie outside the acceptable limits, the results for the patient sample may not be valid.

PERFORMANCE CHARACTERISTICS

Intra-Assay-Precision (N=20) <5% **Inter-Assay-Precision** (N21) <8%

LIMITATIONS OF THE PROCEDURE

The ADI PTH ELISA kit has exhibited no “high dose hook effect” with samples spiked with 1,000,000 pg/mL of Intact PTH. Samples with intact PTH levels greater than the highest calibrator, however, should be diluted and reassayed for correct values. Like any analyte used as a diagnostic adjunct, intact PTH results must be interpreted carefully with the overall clinical presentations and other supportive diagnostic tests.

Expected Values.

Intact PTH levels were measured in fifty-eight (58) apparently normal individuals in the U.S. with the Intact PTH ELISA. The values obtained ranged from 8.8 to 76.6 pg/mL. Based on statistical tests on skewness and kurtosis, the population, when transformed logarithmically, follows the normal or Gaussian distribution as shown in the histograms. The geometric mean + 2 standard deviations of the mean were calculated to be 8.3 to 68.0 pg/mL.

PERFORMANCE CHARACTERISTICS

Accuracy
One hundred twenty-three patient samples, with intact PTH values ranging from 3.2 to 805 pg/mL were assayed by the ELISA procedure and the PTH Immunoradiometric Assay. Linear regression analysis gives the following statistics:

ADI ELISA = 0.997 IRMA Kit + 2.9 pg/mL r = 0.990 N = 123

Sensitivity
The sensitivity, or minimum detection limit, of this assay is defined as the smallest single value, which can be distinguished from zero at the 95% confidence limit.

The ADI PTH ELISA has a calculated sensitivity of 0.9 pg/mL.

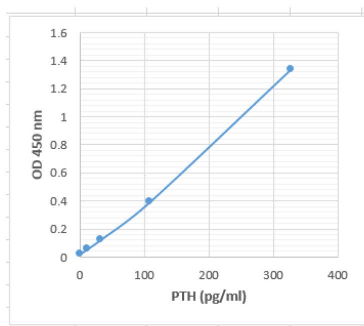
Cross Reactivity

The peroxidase labeled antibody recognizes only the N-terminal region or the 1-34 amino acid sequence of the PTH molecule; whereas the biotinylated antibody is specific to the 39-84 segment. Accordingly, only intact PTH, which requires binding by both the enzyme conjugated and biotinylated antibodies, can be detected by this assay. To further achieve the specificity of this assay, conjugation and biotinylation and the molar ratios thereof, have been optimized to minimize detection of fragments of PTH. Human PTH 1-34 at levels up to 300 pg/mL and the C-terminal 39-84 fragment at levels up to 300,000 pg/mL give molar crossreactivites to PTH of less than 2% and 0.02%, respectively.

WORKSHEET OF A TYPICAL ASSAY

Standard	PTH pg/mL	Mean OD
Standard A	0	0.018
Standard B	12	0.054
Standard C	32.9	0.122
Standard D	109	0.391
Standard E	328	1.338
Standard F	1053	3.960
Control 1	27.6	0.200
Control 2	119	0.799

NOTE: These data are for demonstration purpose only. It must not be used to determine the sample results.



*8_ADI_ELISA

Manual Method

1. For the 450 nm readings, construct a dose response curve (calibration curve) using the first five calibrators provided, i.e. Calibrators A, B, C, D and E. For the 405 nm readings, construct a second dose response curve using the three calibrators with the highest concentrations, i.e. Calibrators D, E and F.
2. Assign the concentration for each calibrator stated on the vial in pg/mL. Plot the data from the calibration curve on linear graph paper with the concentration on the X-axis and the corresponding A.U. on the Y-axis.
3. Draw a straight line between 2 adjacent points. This mathematical algorithm is commonly known as the "point-to-point" calculation. Obtain the concentration of the sample by locating the absorbance unit on the Y-axis and finding the corresponding concentration value on the X-axis. Patient and control samples should be read using the 450 nm for PTH concentrations up to 200 pg/mL. PTH concentrations above 200 pg/mL should be interpolated using the 405 nm reading.

Automated Method:

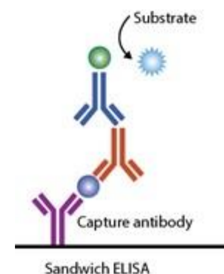
Computer programs using cubic spline or 4 PL [4 Parameter Logistics] can generally give a good fit.

For samples with readout < 200 pg/mL, it is recommended to use the data obtained at 450 nm as shown in **Sample Data** *at 450 nm* in the table above. This practice should give the results with optimum sensitivity of the assay.

Although the readout for Control (2) < 200 pg/mL, it is recommended that the actual result be read out and recorded for quality control evaluation purposes. Further, absorbance for Control 2 is sufficiently high to be analytically valid.

The absorbance readout is off-scale or higher than the average absorbance of the highest calibrator. Sample should be repeated with dilution.

PRINCIPLE OF THE TEST



The Intact PTH Immunoassay is a two-site ELISA [Enzyme-Linked ImmunoSorbent Assay] for the measurement of the biologically intact 84 amino acid chain of PTH. Two different goat polyclonal antibodies to human PTH have been purified by affinity chromatography to be specific for well-defined regions on the PTH molecule. One antibody is prepared to bind only the mid-region and C-terminal PTH 3 9-84 and this antibody is biotinylated. The other antibody is prepared to bind only the N-terminal PTH 1-34 and this antibody is labeled with horseradish peroxidase [HRP] for detection. In this assay, calibrators, controls, or patient samples are simultaneously incubated with the enzyme labeled antibody and a biotin coupled antibody in a streptavidin coated microplate well. At the end of the assay incubation, the microwell is washed to remove unbound components and the enzyme bound to the solid phase is incubated with the substrate, tetramethylbenzidine (TMB). An acidic stopping solution is then added to stop the reaction and converts the color to yellow. The intensity of the yellow color is directly proportional to the concentration of intact PTH in the sample. A dose response curve of absorbance unit vs. concentration is generated using results obtained from the calibrators. Concentrations of intact PTH present in the controls and patient samples are determined directly from this curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5µl, 100µl, 500µl) and multichannel pipet with disposable plastic tips. Bidistilled water, reagent troughs, Orbital shaker, plate washer (recommended) and ELISA plate Reader (450nm).

PRECAUTIONS

Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed. All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken. Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly. All reagents have to be brought to room temperature (18 to 25 °C) before performing the test. Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided. It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions. When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. No reagents from different kit lots have to be used, they should not be mixed among one another. All reagents have to be used within the expiry period. In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision.

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid and BND can be requested or obtained from the ADI website: Sample Diluent and anti-Protein G-HRP contain Proclin 300 (0.05%, v/v). http://4adi.com/objects/catalog/product/extras/ELISA-Kit-SDS-MSDS-Set-1.pdf

SPECIMEN COLLECTION AND HANDLING

The determination of Intact PTH should be performed with EDTA plasma or serum. EDTA plasma has been reported to demonstrate improved PTH stability as compared to serum6. To assay the specimen in duplicate, 50 µL of serum or EDTA plasma is required. Collect whole blood without anticoagulant or lavender [EDTA] tube. After allowing blood to clot, the serum or plasma should be promptly separated, preferably in a refrigerated centrifuge, and stored at -20oC or lower. Serum samples may be stored up to 8 hours at 2-8°C. Serum samples frozen at - 20°C are stable for up to 4 months.

REAGENT PREPARATION: Standards & Controls

Store all kit components at 2-8 oC except Wash Concentrate and Stop Solution upon receipt prior to use

1. All reagents except the calibrators, kit controls and the Wash Concentrate are ready-to-use. Store all reagents at 2-8 oC, except the Wash Concentrate, which should be kept at room temperature until dilution to avoid precipitation.

2. For each of the calibrators (Calibrator A through F) and kit controls 1 and 2, reconstitute each vial with 500 j.tL of Reagent 4 (Reconstitution Solution) and mix. Allow the vial to stand for 10 minutes and then mix thoroughly by gentle inversion to insure complete reconstitution. **Use the calibrators and controls as soon as possible upon reconstitution. *Freeze (-20oC) the remaining calibrators and controls as soon as possible after use.*** Standards and controls are stable at -20 oC for 6 weeks after reconstitution with up to 3 freeze thaw cycles when handled as recommended in "Procedural Notes" section.

3. Reagent A: Wash Concentrate: Mix contents of wash concentrate thoroughly. If precipitate is present in the Wash Concentrate due to storage at lower temperature such as 4oC, dissolve by placing the vial in a 37o C water bath or oven with swirling or stirring. Add wash concentrate (30 mL) to 570 mL of distilled or deionized water and mix. The diluted working wash solution is stable for 90 days when stored at room temperature.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. The unused portions of the standards should be stored at 2-8°C or stored frozen in small aliquots and should be stable for 3 months.

TEST PROCEDURE *(ALLOW <u>ALL REAGENTS</u> TO REACH ROOM TEMPERATURE BEFORE USE).*

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. It is recommended to prepare a parallel replica plates containing all sample for quick transfer to the coated plate.
Place sufficient Streptavidin Coated Strips in a holder to run all six (6) PTH calibrators, A - F of the Intact PTH CALIBRATORS.

Label or mark the microtiter well strips to be used on the plate. Dilute the wash buffer. ***Freeze (-20oC) the remaining stds and controls as soon as possible after use.***

1. Place sufficient **Streptavidin Coated Strips** in a holder to run all six (6) PTH calibrators, A - F of the Intact PTH CALIBRATORS [Exact concentration is stated on the vial label], Quality Control Sera and patient samples.

2. Pipet **25 ul** of sample into the designated or mapped well. ***Freeze (-20oC) the remaining calibrators and controls as soon as possible after use.***

3. Add or dispense **50 ul** of Reagent 1 (Biotinylated Antibody) into each of the wells which already contain the sample.

4. Add or dispense **50 ul** of Reagent 2 (Enzyme Labeled Antibody) into each of the same wells. Cover the microplate(s) with aluminum foil or a tray to avoid exposure to light And place it on an **orbital shaker or rotator** set at 170 + 10 rpm for **3 hours** + 30 minutes at room temperature (22o-28oC).

5. First aspirate the fluid completely and then wash/aspirate each well five (5) times with the Working Wash Solution (prepared from Reagent A), using an automatic microplate washer. The wash solution volume should be set to dispense 0.30-0.35 mL into each well.

6. Add or dispense **150 ul** of the Reagent B (TMB Substrate) into each of the wells.

7. With appropriate cover to avoid light exposure, place the microplate(s) on an **orbital shaker or rotator** set at 170 + 10 rpm for **30 +5 minutes** at room temperature (22o-28oC).

8. Add **100 ul** of the Stop Soln into each of the wells. Mix gently.

9. Read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** against 250 j.tL of distilled or deionized water. **Read** the plate **again** with the reader set to **405 nm** against distilled or deionized water.

Note: The second reading is designed to extend the analytical validity of the calibration curve to the value represented by the highest calibrator, which is approximately 700 – 1,000 pg/mL. Hence, patient samples with PTH > 200 pg/mL can be quantified against a calibration curve consisting of the readings all the way up to the concentration equivalent to the highest calibrator using the 405 nm reading, away from the wavelength of maximum absorbance. In general, patient and control samples should be read using the 450 nm for PTH concentrations up to 200 pg/mL. PTH concentrations above 200 pg/mL should be interpolated using the 405 nm reading.

10. By using the final absorbance values obtained in the previous step, construct a calibration curve via cubic spline, 4 parameter logistics, or point-to-point interpolation to quantify the concentration of the intact PTH.

PROCEDURAL NOTES

- Intact PTH 1-84 is a very labile molecule. Set up the assay immediately upon the reconstitution or the thawing of all calibrators, controls, and patient samples.
- It is recommended that all calibrators, controls, and patient samples are assayed in duplicate. The average absorbance units of duplicate sets should then be used for reduction of data and the calculation of results.
- The samples should be pipetted into the well with minimum amount of air bubble. To achieve this, "reverse pipet" described in the package insert of the manufacturers of Pipettors is recommended.
- Patient samples with values greater than the highest calibrator (Calibrator F), which is approximately 700 – 1,000 pg/mL (see exact concentration on vial label), may be diluted with Reagent 3 (Sample Diluent) and re-assayed. Multiply the result by the dilution factor.
- Reagents from different lot numbers must not be interchanged. □ If preferred, mix in equal volumes, in sufficient quantities for the assay, Reagent 1 (Biotinylated Antibody) and Reagent 2 (Enzyme Labeled Antibody) in a clean amber bottle, Then use 100 j.tL of the mixed antibody into each well. This alternative method should replace Step (3) and (4), to be followed with the incubation with orbital shaker.