INTENDED USE

The Alpha Diagnostics Int'l PTH-biotin ELISA Kit, cat. 0310, is an immunoassay for quantifying PTH-biotin in serum or plasma, or in other appropriately qualified samples from cell culture, bioprocessing solutions, or tissue fluids (e.g., saliva, mucosa). The assay has been specifically validated for quantifying PTH-biotin in dog serum.

GENERAL INFORMATION

Parathyroid hormone (**PTH**) is secreted by the parathyroid gland as an 84 aa (9.4 kDa) peptide. PTH acts to increase the calcium concentration in blood by acting on high density receptors of bone, kidney, CNS, pancreas, testis & placenta. Normal PTH levels in humans is 10 – 60 pg/ml, with a half-life in circulation of about 4 minutes.

PTH conjugated to biotin, [**PTH-biotin**], can be detected in serum separately from unlabeled PTH, and quantified using the ADI ELISA with streptavidin HRP as the detector. The clearance of PTH-biotin in the blood is unknown; it is possible that binding to PTH receptors is possible; also that biotin (B vitamin) may participate in the metabolism of the host, with subsequent clearance and/or other binding properties that may affect detection and quantification.

PRINCIPLE OF THE TEST

The PTH-biotin ELISA kit is based on the binding of PTH-biotin in samples to anti-human PTH antibodies immobilized on the microtiter wells. Streptavidin conjugated to horseradish peroxidase (HRP) enzyme added to the wells binds to the biotin of the bound PTH-biotin. After a washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of PTH-biotin present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of PTH-biotin in samples and control is calculated from a curve of standards containing known concentrations of PTH-biotin.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{o} C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{o}C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

Component	Preparation Instructions
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8^{o}C until the kit lot expires or is used up.
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Streptavidin - HRP Conjugate Concentrate (100x) Part No. S-HRP100, 0.15ml	Peroxidase conjugated streptavidin in buffer with protein, detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8^{o}C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
Anti-Human PTH Microwell Strip Plate	0311	8-well strips (12)	Coated with anti-human PTH, and post-coated with stabilizers.
PTH-biotin Standard Part: 0312	Two (2) vials, each containing PTH-biotin lyophilized in buffer with protein as stabilizers. Keep lyophilized vials refrigerated until used or kit lot expires.		

Freshly reconstitute 1 vial with **1.0** ml **Working Sample Diluent** to provide a **10** ng/ml solution, sufficient for at least two curves. Prepare dilutions, as follows:

Standard	+	Diluent	=	Final Concn
Reconstituted Standard		None		**10** ng/ml
200 ul of 10 ng/ml		**600** ul		**2.5** ng/ml
420 ul of 2.5 ng/ml		**180** ul		**1.75** ng/ml
350 ul of 1.75 ng/ml		**160** ul		**1.2** ng/ml
250 ul of 1.2 ng/ml		**250** ul		**0.6** ng/ml
150 ul of 0.6 ng/ml		**150** ul		**0.3** ng/ml

[**Note**: Use within **2 hours** of preparation]

Immediately freeze the remaining reconstituted 10ng/ml standard for use within 1 week.

TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Streptavidin HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate; 0.2 to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains 1% 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: http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

ASSAY DESIGN AND SET-UP

Sample Collection and Handling
Culture medium, bioprocessing preparations, serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference (See Limits of the Assay, page 6). For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature.

For all samples, clarify by centrifugation and/or filtration prior to dilution in Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

Assay Validation
Validate the performance of the sample antigen and matrix in the assay system for recovery and parallelism (see Limits of the Assay, page 6), as follows:

Recovery – a measure of the interference of the sample matrix (diluent effect) in providing accurate quantitation of the sample PTH-biotin relative to the Standard curve.

Prepare and run a series of dilutions of the sample antigen (concentrations that will fall within the Standard range) in Working Sample Diluent to determine the dilutions that give consistent and accurate quantitation. For most buffer solutions a minimum 5-fold sample dilution is usually sufficient. Serum and plasma require at least a 10-fold dilution to obtain consistent quantitation or complete antigen recovery.

Parallelism – dilutions of the sample should read equivalent values from the top and bottom of the Standard curve to provide good assay precision.

Prepare a dilution series of the sample antigen that gives complete recovery and falls within the full range of the Standard curve. Sample readings from the upper and lower regions of the curve should differ by less than 25%.

Plate Set-up
Bring all reagents to room temperature (18-30^{o} C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of calibrators, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Streptavidin HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

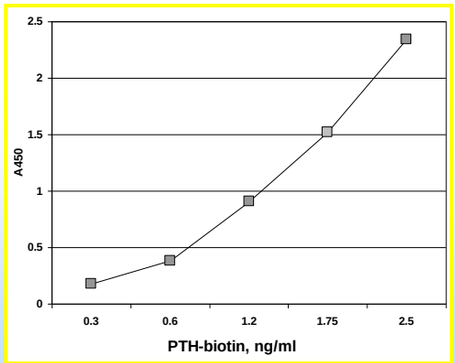
CALCULATION OF RESULTS

1. The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, PTH-biotin concentrations may be determined as follows:
2. Calculate the mean OD of duplicate samples.
3. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of PTH-biotin (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
4. The PTH-biotin concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
5. Multiply the values obtained for the samples by the dilution factor of each sample.
6. Samples producing signals higher than the 2.5 ng/ml standard should be further diluted and re-assayed.

Typical Results:

Wells	Calibrators		A450 nm
A1,2	**Negative Diluent Blank**		0.05
B1,2	0.3 ng/ml	**Calibrator**	0.18
C1,2	0.6 ng/ml	**Calibrator**	0.38
D1,2	1.2 ng/ml	**Calibrator**	0.91
E1,2	1.75 ng/ml	**Calibrator**	1.52
F1,2	2.5 ng/ml	**Calibrator**	2.34
G1,2	Sample	**1:100**	1.45

Sample Result: **1.66** ng/ml x 100 dilution = **166** ng/ml



PERFORMANCE CHARACTERISTICS

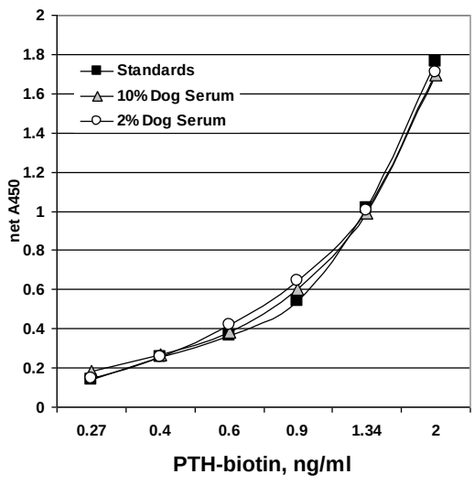
Specificity
The capture antibody used in this kit is specific for human PTH and the detection streptavidin HRP is specific for biotin; therefore, PTH-biotin conjugate is the only analyte measured in the assay. Normal PTH from human, dog or any other source will not be measured.

Precision
Samples containing low and high concentrations of PTH-biotin were assayed as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficients of variation were calculated for the concentrations using a point-to-point curve-fitting program.

PTH-biotin concentrations were measured with good between-assay (1.8 to 10.2 %CV) reproducibility.

Sample	PTH-biotin ng/ml	Inter-assay %CV
High Concentration	2.00	**1.8**
Low Concentration	1.00	**10.2**

Dog Serum: Recovery and Parallelism
PTH-biotin was diluted at 6 concentrations into Sample Diluent containing 2% and 10% dog serum , and assayed in duplicate. Dilution curves are shown in the following graph:



PTH-biotin in 2% dog serum (i.e., dog serum diluted 1/50) and 10% (diluted 1/10) was quantified essentially equivalently to PTH-biotin in Sample Diluent (Standard Curve). Therefore, dilute samples 1/10 or more for accurate quantitation.

QUALITY CONTROL

Reagents Accurate and reproducible assay results rely on proper storage, handling and control of reagent and sample temperature. Store all reagents as indicated, and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

Sample Controls A Positive Serum Control is provided with the kit, assigned with an Humira concentration value range. Recovery in this range is an indicator of proper assay performance. Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Sample Diluent blank should also be run; OD should be <0.3 and lower than 1 ng/ml Standard OD.

Standard Curve The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-uniform or low signals may indicate problems with technique, protocol directions and/or reagent preparation, use or stability. Do not rely on results generated from an assay with these issues.

Technique Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing and handling of samples and reagents.

Equipment Precision of results relies on uniform and effective washing techniques; an automatic washer may be used. ELISA reader and pipettes should be properly calibrated.

LIMITS OF THE ASSAY

1. The **recovery,** or accuracy of PTH-biotin measurement in dog serum (pooled; stored), appears unaffected when diluted at least 1/10 (10%) in Sample Diluent (see Figure on page 6). Recovery in fresh, individual dog serum or plasma samples has not been determined.

2. Normal PTH in serum will bind to the anti-PTH on the coated plate. Normal PTH levels in humans is 10 – 60 pg/ml, which would be 1 – 6 pg/ml when the serum sample is diluted 10-fold or greater. This level is well below the testing range of the assay (300 – 2500 pg/ml) and should not affect test results.
.

Instruction Manual No. M-0310

PTH-biotin (human parathyroid hormone, biotinylated)

ELISA Kit Cat. 0310

For Quantitation of PTH-biotin in Dog Serum

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

ELISA Kit Components	Amount	Part
Anti-Human PTH Coated Plate	8-well strips (12)	0311
PTH-biotin Standard, lyophilized	2 vials	0312
Streptavidin HRP Conjugate (100X)	0.15 ml	S-HRP100
Sample Diluent Concentrate (20x)	10 ml	SD20T
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-0310

DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.