



MicroVue™ Bone

High Sensitivity Human PTH (1-34) EIA

For the quantitative determination of human parathyroid hormone 1-34 levels in plasma or cell culture media

For **Research Use Only**. Not for use in diagnostic procedures.

A symbols glossary can be found at quidel.com/glossary.

INTRODUCTION

Over the years, studies involving the intermittent dosing of human PTH (1-34) in animals and humans have demonstrated an anabolic effect on bone. This effect was positive for bone mass, size, structure, and strength, demonstrating the antifracture efficacy of the treatment. Recently the FDA has approved the recombinant form of human PTH (1-34) – rhPTH (1-34) or teriparatide, for treatment of many forms of osteoporosis. The measurement of circulating levels of this peptide/drug may be useful in assessing its pharmacological properties and for appropriate dosing and schedule optimization. Additionally, this assay may be useful in screening samples for both endogenous levels of circulating hPTH (1-34) and elevated levels of this peptide as it may interfere to varying degrees with 2-site immunometric assays used for measuring intact PTH (1-84).

Obtaining precise measurements of human PTH (1-34) are complicated by antibody cross-reactivity to endogenous PTH (1-84), requiring either a pre-assay immunoextraction of PTH (1-84) or assaying for both PTH (1-34) and intact PTH (1-84) to account for this cross-reactivity.

This High Sensitivity (HS) Human PTH (1-34) ELISA Kit uses two antibodies that have been prepared for optimal recognition of human PTH (1-34) while minimizing binding to human PTH (1-84). We have determined the cross-reactivity of 1-84 to be less than 3% on a molar basis. However, if cross-reactivity correction is desired, the concurrent use of PTH (1-84) standards is suggested.

TEST PRINCIPLE

The High Sensitivity Human PTH (1-34) ELISA Kit is a two-site enzyme-linked immunosorbent assay (ELISA) for the measurement of Human PTH (1-34) in plasma or cell culture media. An affinity purified goat polyclonal antibody optimized to bind human PTH (1-34) is biotinylated for capture. Another affinity purified goat polyclonal antibody, specific to a different area of the human PTH (1-34) peptide, is conjugated with the enzyme horseradish peroxidase (HRP) for detection.

A sample containing human PTH (1-34) is incubated simultaneously with the biotinylated capture antibody and the HRP conjugated detection antibody in a streptavidin coated microliter well. Human PTH (1-34) contained in the sample is immunologically bound by the capture antibody and the detection antibody to form a “sandwich” complex that is further bound to the microtiter well by the streptavidin-biotin complex:

Well/Avidin — Biotin Anti-h PTH — Human PTH (1-34) — HRP Anti-h PTH

At the end of this incubation period, the well is washed to remove any unbound antibody and other components. The enzyme bound to the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microtiter plate reader. The enzymatic activity of the antibody complex bound to the well is directly proportional to the amount of human PTH (1-34) in the sample. A standard curve is generated by plotting the absorbance versus the respective human PTH (1-34) concentration for each standard on linear or logarithmic scales. The concentration of human PTH (1-34) in the samples is determined directly from this curve.

REAGENTS AND MATERIALS SUPPLIED

MicroVue High Sensitivity Human PTH (1-34) EIA Kit contains the following:

Streptavidin Coated Microtiter Plate **40-0010** **1 plate**

Twelve (12) eight well strips and frame (96 wells total). This reagent should be stored in the foil pouch with desiccant at 2°C to 8°C and is stable until the expiration date on the kit.

High Sensitivity Human PTH (1-34) Biotinylated Antibody **40-3910** **2.7 mL**

One (1) vial of biotinylated anti-human PTH in TRIS buffered saline with protein stabilizers and 0.1% Ciprofloxacin as preservative. This reagent should be stored at 2°C to 8°C and is stable until the expiration date on the kit.

High Sensitivity Human PTH (1-34) HRP Antibody **40-3920** **2.7 mL**

One (1) vial of horseradish peroxidase conjugated to anti-human PTH in a stabilized protein solution with 0.1% Ciprofloxacin as preservative. This reagent should be stored at 2°C to 8°C protected from light and is stable until the date on the kit.

NOTE: Make a Working Antibody Solution by pipetting equal volumes of High Sensitivity Human PTH (1-34) Biotinylated Antibody and High Sensitivity Human PTH (1-34) HRP Antibody prior to use. Mix only the volume required for immediate use. Mix well to ensure homogeneity.

High Sensitivity Human PTH (1-34) Standards **40-3931 to 40-3937**

Seven (7) vials each containing human PTH (1-34) lyophilized in a human serum matrix with 0.1% Ciprofloxacin as preservative. **Refer to vial label for exact concentration.** Before use reconstitute the vial with the PTH (1-34) concentration of 0 pg/mL with 2.0 mL of deionized water. Before use reconstitute each of the other six vials of standards with 1.0 mL of deionized water. Allow the vials to sit for approximately 20 minutes with occasional gentle swirling and inversion. Assure complete reconstitution before use.

Use the standards immediately after reconstitution; freeze the unused portion for later use. After reconstitution the standards are stable until the expiration date on the kit when stored at –20°C or below with up to 3 freeze/thaw cycles.

High Sensitivity Human PTH (1-34) Controls I & II **40-3941 and 40-3942**

Two (2) vials each containing human PTH (1-34) lyophilized in a human serum matrix with 0.1% Ciprofloxacin as preservative. Refer to vial label for control ranges. Before use reconstitute each control with 1.0 mL of deionized water. Allow the vials to sit for approximately 20 minutes with occasional gentle swirling and inversion. Assure complete reconstitution before use.

Use the controls immediately after reconstitution; freeze the unused portion for later use. After reconstitution the controls are stable until the expiration date on the kit when stored at –20°C or below with up to 3 freeze/thaw cycles.

- ELISA Wash Concentrate** **40-0041** **20 mL**
 One (1) vial of a 20-fold concentrate. Before use dilute the contents to 400 mL with deionized water and mix well. Upon dilution this yields a working wash solution containing a surfactant in saline with 0.1% Ciprofloxacin as preservative. The diluted wash solution should be stored at room temperature and is stable until the expiration date on the kit.
- ELISA HRP Substrate** **40-0028** **21 mL**
 One (1) bottle of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2°C to 8°C protected from light and is stable until the expiration date on the kit.
- ELISA Stop Solution** **40-0030** **11 mL**
 One (1) bottle of 1 M sulfuric acid. This reagent may be stored at room temperature or at 2°C to 8°C and is stable until the expiration date on the kit.
- Plate Sealer** **10-2016**
 Two (2) included in kit.
- Human PTH Sample Diluent** **10 mL**
(Optional reagent, must be ordered separately using catalog #30-3131)
 One (1) bottle of a lyophilized, treated, stabilized human serum matrix with 0.1% Ciprofloxacin as preservative. Reconstitute with 10 mL of deionized water. See insert accompanying product for preparation and storage.

MATERIALS REQUIRED BUT NOT SUPPLIED IN KIT

- 1.0 mL volumetric pipette for reconstituting standards and controls
- Precision pipets capable of delivering 50 µL and 100 µL
- Aluminum foil
- Automated microtiter plate washer OR
- Repeating dispenser for delivering 350 µL and suitable aspiration device
- Container for storage of wash solution
- Spectrophotometric microtiter plate reader capable of reading absorbance at 450 nm and at 595-650 nm
- Deionized water
- Horizontal rotator capable of maintaining 180-220 RPM
- Timer

WARNINGS AND PRECAUTIONS

- Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid (i.e. ELISA HRP Substrate and ELISA Stop Solution). In case of contact with any of these reagents, wash thoroughly with water. TMB is a suspected carcinogen. Use Good Laboratory Practices. Wash hands before eating. Do not eat, drink or smoke in the work area.
- **CAUTION: Potential Biohazardous Material**
 HANDLE ASSAY REAGENTS AS IF CAPABLE OF TRANSMITTING AN INFECTIOUS AGENT.
- The human source material used in the preparation of this product has been tested by an FDA approved method for the presence of antibodies to Human Immunodeficiency Virus (HIV I and HIV II) and to Hepatitis C virus (HCV), as well as for Hepatitis B surface antigen (HBsAg) and found to be negative. Because no test method can offer complete assurance that HIV I and HIV II, HCV, HBsAg or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human urine, serum or blood specimen in the Centers for Disease Control/National Institutes of Health Manual "Biosafety in Microbiological and Biomedical Laboratories," 1993.
- Testing should be performed in an area with adequate ventilation.

- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

Preparation and Storage

Store the kit at 2°C to 8°C upon receipt. **Store the standards and controls at –20°C or below after reconstitution.** 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 and mix by gentle swirling and inversion.

Reagents from different kit lot numbers should not be combined or interchanged.

SPECIMEN COLLECTION

Measurement of the PTH (1-34) concentration should be made using EDTA plasma or cell culture media. Three hundred microliters * (**See Modifications**) of plasma or media are required to assay the sample in duplicate. Centrifuge the sample and separate the plasma or media from the cells. Samples should be assayed immediately or stored frozen at –20°C or below. Avoid repeated freezing and thawing of specimens.

ASSAY PROCEDURE

1. Place a sufficient number of Streptavidin Coated Strips in a holder to run PTH standards, controls and unknown samples.
2. Pipet 150 µL* of standard, control, or sample into the designated or mapped well. Freeze the remaining standards and controls as soon as possible after use.
3. Pipet 50 µL of the Working Antibody Solution consisting of 1-part HRP Antibody and 1-part Biotinylated Antibody into each well.
4. Cover the plate with one plate sealer, then cover with aluminum foil to avoid exposure to light.
5. Incubate plate at room temperature for three (3) hours on a horizontal rotator set at 180-220 RPM.
6. Remove the aluminum foil and plate sealer. **Using an automated microtiter plate washer aspirate the contents of each well. Wash each well five times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents.** A suitable aspiration device may also be used.
7. Pipet 200 µL* of ELISA HRP Substrate into each of the wells.
8. Re-cover the plate with the plate sealer and aluminum foil. Incubate at room temperature for 30 minutes on a horizontal rotator set at 180-220 RPM.
9. Remove the aluminum foil and plate sealer. Read the absorbance at 620 nm (see Note) within 5 minutes in a microtiter plate reader against the 0 pg/mL Standard wells as a blank.
10. Immediately pipet 50 µL of ELISA Stop Solution into each of the wells. Mix on horizontal rotator for 1 minute.
11. Read the absorbance at 450 nm within 10 minutes in a microtiter plate reader against a reagent blank of 200 µL* of Substrate and 50 µL of Stop Solution.

If dual wavelength correction is available set the Measurement wavelength to 450 nm and Reference wavelength to absorbance used in step #9.

NOTE: Absorbance may be read at wavelengths from 595 nm to 650 nm depending upon available filters.

* See Modifications

PROCEDURAL NOTES

- It is recommended that all standards, controls and samples be assayed in duplicate. The average absorbance reading of each duplicate should then be used for data reduction and the calculation of results.
- Keep light sensitive reagents (i.e. HRP Antibody, the Working Antibody Solution consisting of combined HRP Antibody and Biotinylated Antibody, and ELISA HRP Substrate) in the original amber bottles or other suitable container which is well protected from light.
- Store any unused Streptavidin Coated Strips in the resealable aluminum pouch with desiccant to protect from moisture.
- The sample and all reagents should be pipetted carefully to minimize air bubbles in the wells.
- The sequence and timing of each reagent addition is important as both the immunological and enzymatic reactions are in kinetic modes. The washing step is also an important part of the total assay procedure. **The use of an automated microtiter plate washer is strongly recommended.** All pipetting and washing steps should be performed such that the timing is as consistent as possible.
- Samples with values greater than the highest standard should be diluted 1:10 with the 0 pg/mL Standard and reassayed. Multiply the result by 10. (See Limitations, # 2)
- Plasma or cell culture media samples may contain fibrin clots or cellular debris. Freeze/thaw of plasma samples may accelerate clot formation. These samples must be centrifuged and decanted prior to assay to remove all particulate material which can cause random high non-specific binding on well surface.
- Rarely, upon opening the streptavidin plate, small white crystals may be observed in some of the wells. This is entirely cosmetic and will not affect the assay. This condition is reported by other kit manufacturers and results from the final stabilizing buffer used in the coating process.

CALCULATION OF RESULTS

The two absorbance readings taken before and after the addition of the ELISA Stop Solution allow for the construction of two standard curves using the human PTH 1-34 standards contained in the kit. **Refer to the individual vial label for exact concentration.** The primary curve used for calculation of results is the second reading taken after the addition of the ELISA Stop Solution and read at 450 nm. This data utilizes the absorbance values obtained with the first six standards. The first reading taken before the addition of the ELISA Stop Solution and read at 595 nm-650 nm is intended to extend the analytical range to the value of the seventh (highest) standard provided in this kit. **It should be used only for sample results that fall between the value of the sixth and seventh standard.** Results obtained with the first reading should not replace the on-scale reading at 450 nm. Each curve should be generated as follows:

Primary Procedure—Read at 450 nm

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

The PTH 1-34 concentration of the samples is read directly from the standard curve using their respective corrected absorbance. If log-log graph paper or computer assisted data reduction programs utilizing logarithmic transformation are used, samples having corrected absorbance between the 0 pg/mL Standard and the next highest standard should be calculated by the formula:

$$\text{Value of unknown} = \frac{\text{Corrected Absorbance (unknown)}}{\text{Corrected Absorbance (2}^{\text{nd}} \text{ Std.)}} \times \text{Value of the 2}^{\text{nd}} \text{ Std.}$$

Secondary Procedure—Read at 595 nm to 650 nm

1. Calculate the average absorbance for each pair of duplicate assay wells.
2. The standard curve is generated by plotting the absorbance of the three highest standards on the ordinate against the standard concentration on the abscissa using linear-linear or log-log graph paper.
3. The human PTH 1-34 concentration of samples reading only between the sixth and seventh standard are read directly from this standard curve.

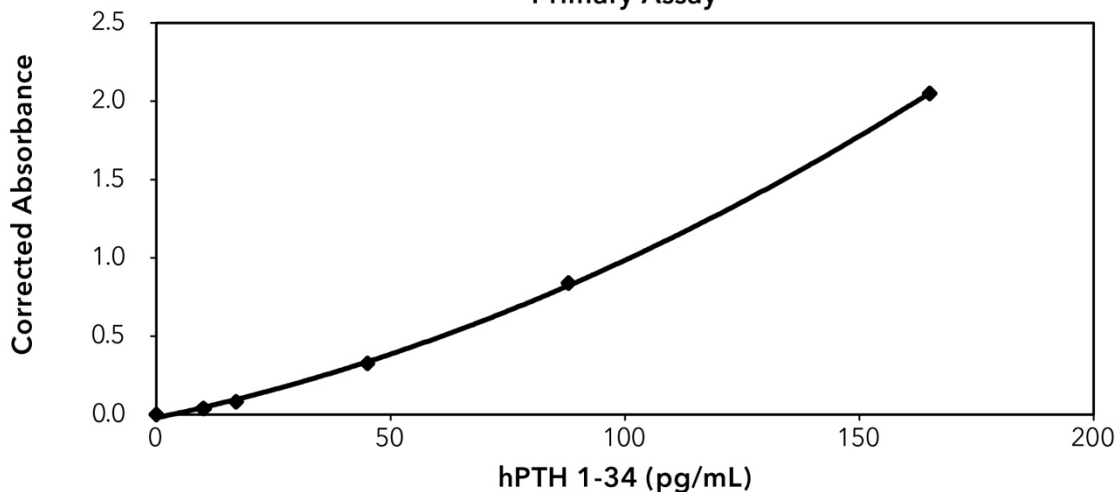
EXAMPLE DATA AND STANDARD CURVE

The following are representative examples of data and the resulting standard curves from the primary and secondary procedures. **These curves should not be used in lieu of a standard curve run with each assay.**

Primary Assay—450 nm				
Well I.D.	ABS	Average ABS	Corrected ABS	Results pg/mL
Reagent Blank	0.000 0.000	0.000		
0 pg/mL	0.018 0.020	0.019	0.000	
10 pg/mL	0.059 0.056	0.057	0.038	
17 pg/mL	0.098 0.100	0.099	0.080	
45 pg/mL	0.342 0.348	0.345	0.326	
88 pg/mL	0.851 0.865	0.858	0.839	
165 pg/mL	2.066 2.071	2.068	2.049	
Control I	0.154 0.137	0.145	0.126	23
Control II	1.526 1.538	1.532	1.513	132
Sample 1	3.186 3.094	3.140	3.121	*

* >165 pg/mL; Calculate using secondary assay.

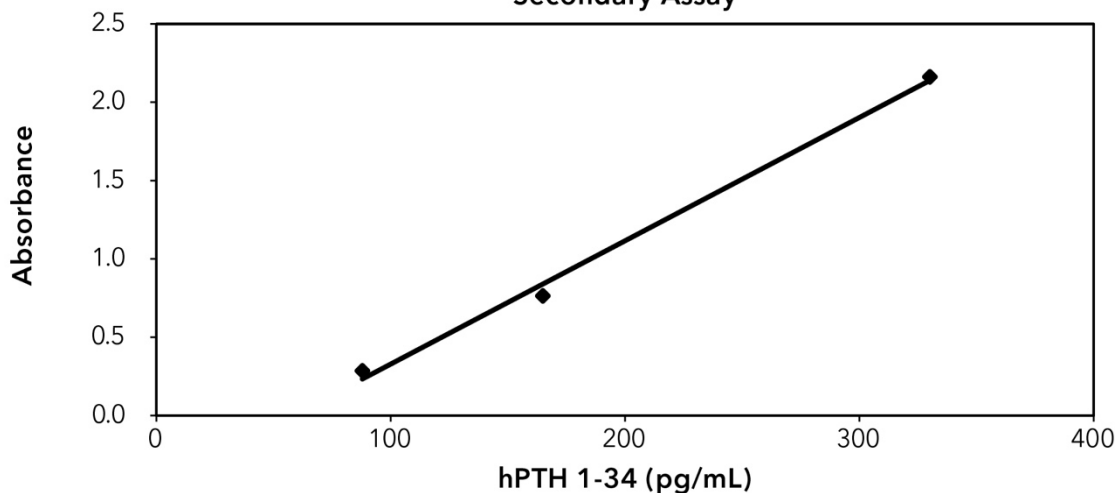
**High Sensitivity Human PTH 1-34 ELISA
Primary Assay**



Secondary Assay—620 nm

Well I.D.	ABS	Average ABS	Results pg/mL
0 pg/mL	0.009	0.012	
	0.014		
88 pg/mL	0.281	0.286	
	0.290		
165 pg/mL	0.752	0.764	
	0.776		
330 pg/mL	2.150	2.163	
	2.176		
Sample 1	1.152	1.110	207
	1.068		

**High Sensitivity Human PTH 1-34 ELISA
Secondary Assay**



LIMITATIONS OF THE PROCEDURE

- The lowest concentration of human PTH (1-34) measurable is 0.9 pg/mL* (assay sensitivity) and the highest concentration of human PTH (1-34) measurable without dilution is the value of the highest standard. **(See Modifications)**
- The reagents in this High Sensitivity Human PTH (1-34) ELISA Kit have been optimized so that the high dose “hook effect” is not a problem for samples with elevated PTH (1-34) values. Samples with levels between the highest standard and 800,000 pg/mL will read greater than the highest standard and should be diluted 1:10 or more with the 0 pg/mL Standard and reassayed for correct values.
- Grossly lipemic plasma samples may affect the immunological response and it is recommended that results obtained with such samples be scrutinized accordingly.
- Differences in protein concentration and protein type between samples and standards in an immunoassay contribute to "protein effects" and dose biases. When measuring low protein concentration culture media samples against high protein concentration standards, it is recommended that like samples be assayed together in the same assay to minimize this bias.

QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known levels of human PTH (1-34). MicroVue recommends that all assays include the laboratory's own human PTH (1-34) controls in addition to those provided with this kit.

PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity of the High Sensitivity Human PTH (1-34) ELISA assay as determined by the 95% confidence limit on 20 duplicate determinations of the 0 pg/mL Standard is 0.9 pg/mL.* **(See Modifications)**

Precision

To assess intra-assay precision the mean and coefficient of variation were calculated from 20 duplicate determinations of two samples each performed in a single assay.

Mean Value (pg/mL)	Coefficient of Variation
22.3	2.6%
127	2.1%

To assess inter-assay precision the mean and coefficient of variation were calculated from duplicate determinations of two samples performed in 11 assays.

Mean Value (pg/mL)	Coefficient of Variation
23.1	8.5%
130	6.2%

Cross-reactivity

The following cross-reactants were diluted in the 0 pg/mL Standard and measured using the High Sensitivity Human PTH (1-34) ELISA Kit. The results are expressed as % cross-reactivity relative to the human PTH (1-34) standards contained in the kit.

Cross-reactant Measured	Cross-reactivity:	
	Weight Basis	Molar Basis
human PTH (1-34)	100.0	100.0
human PTH (1-84)	6.5	2.8
human PTH (7-84)	<0.001	<0.0004
rat PTH (1-34)	43.0	43.6
rat PTH (1-84)	6.8	3.0

STANDARDIZATION

The standards in this High Sensitivity Human PTH 1-34 ELISA Kit are dose assigned versus the WHO International Standard, Parathyroid Hormone 1-34, Recombinant, Human, NIBSC code: 04/200 (www.nibsc.ac.uk).

MODIFICATIONS

When sampling from rats or mice it is often necessary to use a smaller sample volume.

This assay can be easily modified to use either a 25 μ L or a 50 μ L sample size by lowering the volume of standard, control and sample pipetted in Step #2 of the Assay Procedure **AND** reducing the volume of ELISA HRP Substrate pipetted in Step #7 to 100 μ L. All other procedural steps remain the same.

Reducing the sample size will change the reported sensitivity. As determined by the 95% confidence limit on 20 duplicate determinations of the 0 pg/mL Standard, the sensitivity for each sample size is as follows:

25 μ L	50 μ L	150 μ L
5.8 pg/mL	2.5 pg/mL	0.9 pg/mL

ASSISTANCE

To place an order or for technical support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at (740) 592-9820. For e-mail support contact customerservice@quidel.com or technicalsupport@quidel.com.

For services outside the U.S.A., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website quidel.com.

REFERENCES

1. Hämmerle SP, Mindeholm L, Launonen A, Kiese B, Loeffler R, Harfst E, Azria M, Arnold M, John M, "The single dose pharmacokinetic profile of a novel oral human parathyroid hormone formulation in healthy postmenopausal women", *Bone*, Vol. 50, 2012; 965-973.
2. Reeve J, "Teriparatide [rhPTH (1-34)] and Future Anabolic Treatments in Osteoporosis", In: Favus MJ, Ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. Washington, D.C.; American Society for Bone and Mineral Research, Sixth Edition, 2003; 344-349.
3. Gao P, Schmidt-Gayk H, Dittrich K, Nolting B, Maier A, Roth H J, Seemann O, Reichel H, Ritz E, Schilling T, "Immunochemiluminometric assay with two monoclonal antibodies against the N-terminal sequence of human parathyroid hormone", *Clinica Chimica Acta*, Vol. 245, 1996; 39-59.

REF

60-3900 MicroVue High Sensitivity Human PTH (1-34) EIA – 96 Test

RUO



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PI60390001EN00 (11/19)

GLOSSARY

REF

Catalogue number

LOT

Batch code



Use by



Manufacturer



Temperature limitation



Consult e-labeling instructions for use

RUO

For Research use only



Contains sufficient for 96 determinations

CONT

Contents/Contains

CONTROL

Control
