

An enzyme immunoassay for the determination of human osteoprotegerin in experimental samples

For Research Use Only

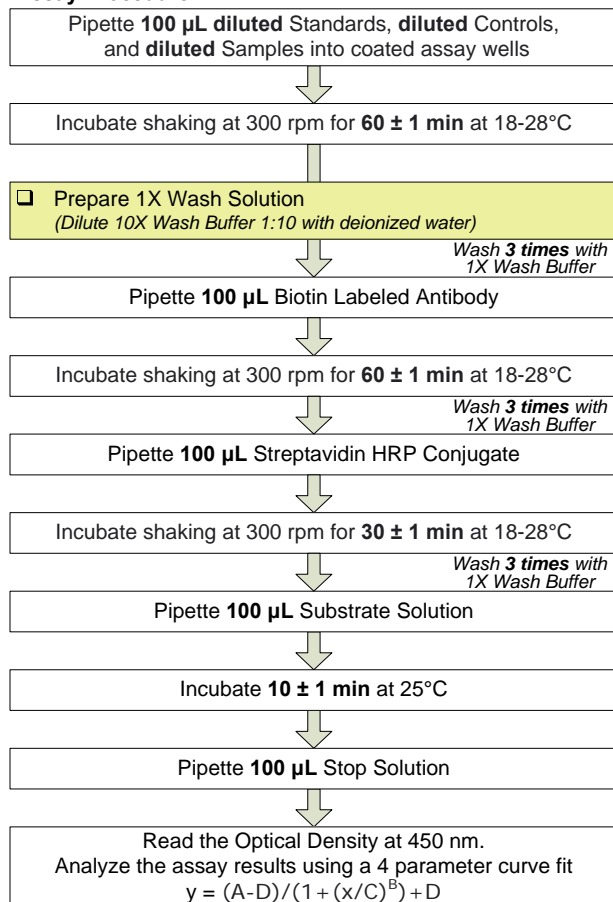
Not for Use in Diagnostic Procedures

MicroVue™ OPG EIA Summary

Reagents and Samples Preparation

- Reconstitute Standard with Dilution Buffer (*see C of A*)
- Prepare serial dilutions of Standards according to Table 1.
- Reconstitute Controls with Deionized water (*see C of A*)
- Dilute Controls and Samples 1:3 with Dilution Buffer (e.g. 100 µL sample + 200 µL Dilution Buffer) **Caution:** Use **only** polypropylene or polyethylene tubes for this dilution step.

Assay Procedure



PURPOSE OF THE TEST

The MicroVue OPG assay is an enzyme immunoassay for the quantitative measurement of total osteoprotegerin in plasma, serum, or other experimental sample types. This assay is intended for research use only.

SUMMARY AND EXPLANATION

Osteoprotegerin (OPG) or osteoclastogenesis inhibitory factor (OCIF) is a secretory glycoprotein belonging to the TNF super family. OPG consists of 401 amino acid residues; it has a molecular weight of 60 kDa as a monomer and 120 kDa as a disulfide-linked dimer and is produced in a wide range of tissues, e.g. bone, skin, liver, stomach, intestine, and lung. Osteoprotegerin inhibits the binding of RANK to RANKL (TRANCE, osteoprotegerin ligand, OPGL, osteoclast differentiation factor, ODF), thus inhibiting the recruitment, proliferation and activation of osteoclasts. Since OPG exhibits an inhibitory effect on osteoclasts, it acts as a soluble factor in the regulation of bone mass.^{1,2,3} Osteoclast formation activity may be the cause of bone loss in many imbalances in bone metabolism such as osteoporosis, osteopetrosis, hypercalcemia, metastatic osteolytic lesions and rheumatic bone degradation.^{2,4}

PRINCIPLE OF THE PROCEDURE

In the MicroVue Osteoprotegerin ELISA, the Standards, Controls and specimens are incubated in duplicate assay wells coated with a murine monoclonal anti-human osteoprotegerin antibody. After a 60-minute incubation, unbound material is removed by a washing step, and a solution containing a biotin-labeled polyclonal anti-human osteoprotegerin antibody is added. After a 60 minute incubation, and a second washing step to remove unbound antibody, a streptavidin horseradish peroxidase conjugate is added to the assay wells. The conjugate is incubated for 30 minutes, a final round of washing removes unbound material, and the remaining conjugate is allowed to react with the substrate consisting of tetramethylbenzidine (TMB). The reaction is stopped by the addition of an acidic solution, and the optical density of the resulting yellow product is measured at 450 nm. The absorbance is proportional to the concentration of osteoprotegerin in the well. A standard curve is constructed by plotting absorbance value versus osteoprotegerin concentrations of Standards. Concentrations of unknown samples are determined using this standard curve.

REAGENTS AND MATERIALS PROVIDED

39 Assays for OPG conducted in duplicate (96 wells total)

MicroVue OPG Assay kit contains the following:

- S Human OPG Standard Item C043541 1 each**
Lyophilized recombinant chimeric protein composed of Human OPG and Fc domain of Human IgG containing 0.2% Methylisothiazolone and 0.1% ProClin® 950 as preservatives; The exact value is stated on each vial
- L Low/High Controls Items C044261, C044161 1 each**
Lyophilized human OPG isolated from human serum containing 0.2% Methylisothiazolone and 0.1% ProClin 950 as preservatives; **Refer to the Certificate of Analysis for acceptable ranges**
- 1 Coated Strips Item C041212 12 each**
Purified murine monoclonal Anti-OPG antibody adsorbed onto stripwells
- 2 Stop Solution Item C008111 13 mL**
Contains 1.96% Sulfuric Acid
- 3 10X Wash Buffer Item C006121 100 mL**
Contains < 0.05% Thimerosal as a preservative
- 4 Dilution Buffer Item C005111 13 mL**
Contains < 0.05% Thimerosal as a preservative
- 5 Biotin-Labeled Anti-human Osteoprotegerin Antibody Item C042511 13 mL**
Anti-OPG antibody conjugated to Biotin in a buffered solution containing stabilizers and < 0.05% Thimerosal as a preservative; Ready-to-use
- 6 Substrate Solution Item C007111 13 mL**
Tetramethylbenzidine (TMB), < 0.05% and Hydrogen Peroxide, 0.01%; Ready-to-use
- 7 Streptavidin-Horseradish Peroxidase Conjugate Item C042311 13 mL**
Streptavidin protein conjugated to Horseradish Peroxidase-containing buffer salts and stabilizers with Methylisothiazolone, Bromonitrodiodioxan and ProClin 300, 0.02% each, as preservatives. Ready to use.

ProClin® is a registered trademark of Rohm and Haas Company.

MATERIALS REQUIRED BUT NOT PROVIDED

- Adjustable precision micropipettes for dispensing 50 - 1000 µL volumes with disposable tips
- Multichannel pipette capable of dispensing 100 µL volumes with disposable tips
- Polypropylene or polyethylene test tubes for diluting Standards, Controls and Samples
- Microplate shaker capable of constant shaking at 300 rpm for 60 minutes
- Labware suitable for liquid measurement of 10-1000 mL
- Deionized or distilled water
- Microplate reader with a 450 nm filter
- Software package facilitating data generation and data analysis with a four-parameter curve fit
- Suitable device for washing the microplate
- Absorbent material for blotting the in-process microplate after washing
- Computer with CD/DVD ROM Drive

WARNINGS AND PRECAUTIONS

- For Research Use Only. Not for Use in Diagnostic Procedures.
- Treat specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any patient samples. Wear gloves and safety glasses when handling specimen samples and kit reagents.
- Wear suitable protective clothing, gloves, and eye/ face protection when handling contents of this kit.
- Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
- Store assay reagents as indicated.
- Do not use Coated Strips if pouch is punctured.
- Thimerosal is used as a preservative. Incidental contact with or ingestion of buffers or reagents containing Thimerosal can lead to increased hypersensitivity reactions including irritation to the skin, eyes, or mouth. Seek medical attention if symptoms are experienced. Exposure to Thimerosal may have potential mutagenic effects.
- ProClin® 300 & 950 are used as a preservative. Incidental contact with or ingestion of buffers or reagents containing ProClin can cause irritation to the skin, eyes or mouth. Use good laboratory practices to reduce exposure. Seek medical attention if symptoms are experienced.
- The Stop Solution contains Sulfuric Acid and may cause irritation. Do not ingest. Avoid contact with skin, eyes, or clothing, and wear gloves and safety glasses when handling. Always avoid contact with strong acids and bases. If contact is made, immediately rinse thoroughly with water. If ingested, contact a physician.
- This kit contains components of human origin. These materials were tested by an FDA-approved method for the presence of antibody to human immunodeficiency virus (HIV1 and HIV2) and to hepatitis C virus, as well as for hepatitis B surface antigen. Since no test method can offer complete assurance that infectious agents are absent, these reagents should be handled at Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 2007.
- Use of multichannel pipettes or repeater pipettes is recommended to ensure the timely delivery of reagents.
- For accurate measurement of samples, add samples and Standards precisely. Pipette carefully, preferably in contact with the side of the Assay Wells at a 45° angle, using only calibrated equipment.
- Always use clean lab ware when performing this assay.
- Do not use glass tubes.
- Avoid cross contamination of samples by always using new pipette tips.
- Test each sample in duplicate.

17. Do not use a microassay well for more than one test.
18. Using incubation times and temperatures other than those indicated in the *ASSAY PROCEDURE* section may give erroneous results.
19. Mix reagents thoroughly prior to application to Assay Wells.
20. Add 100 µL of Dilution Buffer to any empty wells in order to reduce potential edge effects.
21. Substrate Solution is light sensitive. Avoid prolonged exposure to direct sunlight.
22. Do not let wells dry out once the assay has begun.
23. When adding or removing liquid from the microassay wells, do not scrape or touch the bottom of the wells.
24. Perform this assay with any validated washing method. Do not wash wells with a multi-channel pipette.
25. Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.

REAGENT PREPARATION

All reagents should be equilibrated to 18 – 28°C prior to use. Prepare assay reagents as follows:

Standard

Reconstitute the lyophilized Standard with Dilution Buffer to prepare stock solution with a concentration of 60 pmol/L. Refer to the Certificate of Analysis included in the kit for the current reconstitution volume required. Mix the stock gently and incubate at room temperature for 15 minutes to ensure complete reconstitution. Always avoid foaming when reconstituting or mixing the protein solution.

Standard Curve Preparation

Subsequent concentration levels are to be prepared as follows:

Table 1: Preparation of Standards

Std. #	Dilution Buffer Volume	Standard Volume	Relative Concentration
Std 1	600 µL	300 µL of stock	60 pmol/L
Std 2	300 µL	300 µL of Std 1	30 pmol/L
Std 3	300 µL	300 µL of Std 2	15 pmol/L
Std 4	300 µL	300 µL of Std 3	7.5 pmol/L
Std 5	300 µL	300 µL of Std 4	3.75 pmol/L
Std 6	300 µL	300 µL of Std 5	1.875 pmol/L

Standard stock solution (60 pmol/L) should be aliquoted and frozen at ≤ -20°C until next use. Avoid repeated freeze/thaw cycles.

NOTE: Only polypropylene or polyethylene tubes should be used for preparation of standards. **Do not use glass tubes!**

Do not store the prepared standards 1-6.

Controls

Reconstitute the lyophilized Controls with deionized water. Refer to the Certificate of Analysis included in the kit for the current reconstitution volume required. Mix gently, and incubate at room temperature for 30 minutes to ensure complete reconstitution.

Dilute Controls prior to use in the assay 1:3 using Dilution Buffer (e.g. 100 µL Control + 200 µL Dilution Buffer for duplicates).

Reconstituted Controls should be aliquoted and frozen at ≤ -20°C until next use. Avoid repeated freeze/thaw cycles.

Wash Solution

Dilute 100 mL of Wash Solution Concentrate (10X) with 900 mL of deionized (distilled) water. The diluted Wash Solution is stable for 1 month when stored at 2-8°C.

STORAGE

Store the kit at 2–8°C. Store unused reagents at 2–8°C. Under these conditions, assay components are stable until the expiration date printed on the box label.

Human Osteoprotegerin Standard

Standard stock solution (60 pmol/L) should be aliquotted and frozen at ≤ -20°C until next use. Avoid repeated freeze/thaw cycles.

Do not store the diluted Standard solutions.

Controls

Reconstituted Controls should be aliquoted and frozen at ≤ -20°C until next use. Avoid repeated freeze/thaw cycles.

Do not store the diluted (1:3) Controls.

SPECIMEN COLLECTION AND STORAGE

Serum and plasma (Citrate, Heparin, and EDTA) have been used as samples in the MicroVue OPG Assay. Collect specimens using standard venipuncture techniques. Specimens should be collected in such a way to avoid hemolysis. For serum specimens, allow the blood to clot, and separate the serum by centrifugation. Both Heparin and EDTA plasma can be used. See *OBSERVED VALUES* section for more information.

Stability of Samples at 4°C

Samples should be stored at ≤ -20°C. However, no decline in sample concentration of OPG has been observed in plasma or serum samples stored at 4°C for 2 weeks.

Mean values of OPG in serum, citrate plasma, EDTA plasma and heparin plasma after 7 and 14 days at 4°C (expressed as pmol/L):

Incubation	-	7 days	14 days
Temperature	-20°C	4°C	4°C
Sample (n=5)	pmol/L	pmol/L	pmol/L
Serum	7.54	7.84	7.62
EDTA Plasma	7.50	7.66	7.31
Citrate Plasma	6.55	6.46	6.46
Heparin Plasma	7.85	7.81	7.68

Samples can be stored up to 8 hours at room temperature, up to 14 days at 4°C, or at ≤ -20°C for long-term storage. Do not subject samples to more than 3 freeze/thaw cycles.

Heparin plasma samples are not stable for multiple freeze/thaw cycles and should be aliquotted in individual vials to avoid freeze/thaw damage.

Samples (n=3)	# Freeze / thaw cycles		
	1x pmol/L	3x pmol/L	5x pmol/L
Serum	8.47	7.91	7.45
EDTA Plasma	8.21	7.75	7.07
Citrate Plasma	6.81	6.82	6.29
Heparin Plasma	7.45	6.41	6.25

ASSAY PROCEDURE

Read entire product insert before beginning the assay.

See *WARNINGS AND PRECAUTIONS* and *REAGENT PREPARATION*.

Sample Incubation

1. Prepare standard curve as described in *REAGENT PREPARATION*.
2. Dilute Controls and Specimens 1:3 with Dilution Buffer (e.g. 100 µL sample + 200 µL Dilution Buffer). Only polypropylene or polyethylene tubes should be used for control and specimen dilution. **Do not use glass tubes!**
3. Allow pouch of Coated Strips to equilibrate to 18 - 28°C before opening. Remove Stripwell Frame and the required number of Coated Strips from the pouch. Ensure that the pouch containing any unused strips is completely resealed and contains desiccant.
4. Place desired number of Coated Strips in the Stripwell Frame. Label strips to prevent mix-up in case of accidental removal from the frame.
5. Pipette 100 µL of diluted Standards, Controls, Specimens, and Dilution Buffer (Blank) in duplicate into the appropriate wells. See Table 2 for an example of assay template.

Table 2: Assay Template

#	Strips 1&2	Strips 3&4	Strips 5&6	Strips 7&8	Strips 9&10	Strips 11&12
A	Blank	High	Sample 8	Sample 16	Sample 24	Sample 32
B	Standard # 1	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	Standard # 2	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	Standard # 3	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	Standard # 4	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	Standard # 5	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	Standard # 6	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	Low	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

6. Incubate plate at 25°C for 60 ± 1 minutes while shaking at 300 rpm on an orbital microplate shaker.

Wash Cycle

7. After incubation, wash the microplate wells three times using 1X Wash Solution (a minimum of 300 µL per well). Be sure that wells are aspirated completely between washes. After washing, tap the wells on a paper towel to expel any remaining liquid. Do not let wells dry between steps.

Antibody Incubation

8. Add 100 µL of Biotin-Labeled Anti-Osteoprotegerin Antibody Solution to each well.
9. Incubate the plate at room temperature for 60 ± 1 minutes while shaking at 300 rpm on an orbital microplate shaker.
10. After incubation, wash the microplate wells according to the Wash Cycle section above.

Conjugate Incubation

11. Add 100 µL of Streptavidin-HRP Conjugate Solution to each well.
12. Incubate the plate at room temperature for 30 ± 1 minutes while shaking at 300 rpm on an orbital microplate shaker.
13. After incubation, wash the microplate wells according to the Wash Cycle section above.

Substrate Incubation

14. Pipette 100 µL of Substrate Solution into each well. (Avoid exposing the microtiter plate to direct sunlight. Covering the entire plate using aluminum foil is recommended).
15. Incubate the plate for 10 minutes at 25°C.

Stop/Read

16. Stop the color development of the reaction by pipetting 100 µL of Stop Solution into each well. Assure that no large bubbles are present in the wells and that the bottoms of the strips are clean and dry.
17. Determine the absorbance of each well at 450 nm. The absorbance must be read within **5 minutes** of adding the Stop Solution.
18. Quantitation software with a 4-parameter calibration curve fitting equation must be used to analyze the MicroVue OPG assay results.

$$\text{Equation: } y = (A-D)/(1+(x/C)^B)+D$$

QUALITY CONTROL

The Certificate of Analysis included in this kit is lot specific and is to be used to verify that the results obtained by your laboratory are similar to those obtained by Quidel Corporation. The optical density values provided are intended as a guideline only. The results obtained by your laboratory may differ.

Quality Control ranges are provided. The control values are intended to verify the validity of the curve and sample results. Each laboratory should establish its own parameters for acceptable assay limits. If the control values are NOT within your laboratory's acceptance limits, the assay results should be considered questionable and the assay should be repeated.

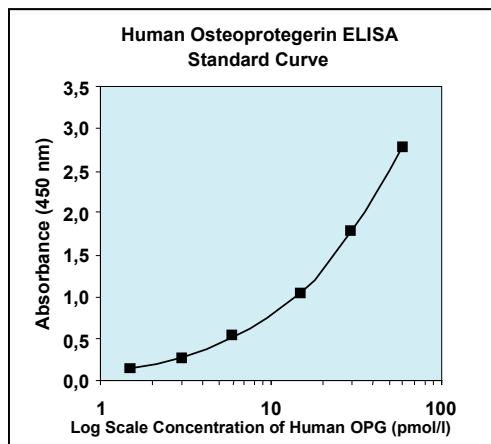
Samples that result in an OPG level that exceeds 60 pmol/L should be re-tested using greater dilutions. Dilution factors will need to be taken into consideration in calculating the final osteoprotegerin concentration of a sample in this situation.

INTERPRETATION OF RESULTS

Most Microtiter plate readers perform automatic calculations of analyte concentration. The calibration curve is constructed by plotting absorbance (Y) of Standards versus the known concentration (X) of Standards using the four-parameter function. See the representative Standard Curve.

The dilution buffer suppresses the matrix effect of the samples. The concentrations of samples can be read directly off the curve without considering dilution factors. Dilution factors must be taken into account when calculating results for samples that are diluted greater than 1:3. For example, results for a sample diluted 1:6 must be multiplied by 2 to accommodate the dilution factor (1:3 x 2(DF) = 1:6).

Representative Standard Curve



NOTE: The 1:3 dilution of the Controls and Specimens is accounted for in the preparation of the Standards; therefore, inclusion of a dilution factor is unnecessary.

OBSERVED VALUES

The mean value of OPG has been estimated by ELISA using serum samples from apparently healthy donors (n = 29, mean ± SEM):

$$5.7 \pm 0.42 \text{ pmol/L}$$

It is recommended that each individual laboratory establish its own normal control range. The reference range provided above should be regarded as a guideline only.

Effect of sample matrix (serum/plasma)

Samples from 10 volunteers were taken and treated by different methods. The results are summarized below:

Volunteer	Serum (pmol/L)	Plasma (pmol/L)		
		EDTA	Citrate	Heparin
1	8.47	9.52	7.97	8.37
2	5.42	4.89	4.47	4.95
3	6.81	6.86	6.61	6.23
4	10.99	12.48	10.25	10.35
5	8.68	9.57	7.95	9.57
6	6.11	5.66	6.46	5.72
7	6.96	7.59	6.95	7.29
8	8.29	8.07	6.70	7.27
9	7.85	8.10	6.73	7.13
10	9.59	8.49	7.00	7.97

Mean values of OPG in serum, citrate plasma, EDTA plasma and heparin plasma (expressed as pmol/L):

Sample (n=10)	Mean (pmol/L)	Plasma/Serum (%)
Serum	7.92	-
EDTA Plasma	8.12	102.6
Citrate Plasma	7.11	89.8
Heparin Plasma	7.49	94.5

PERFORMANCE OF THE TEST

This section details the analytical data of the MicroVue OPG Assay. For actual standard curve and quality control values see the Certificate of Analysis for each kit lot.

Sensitivity

The limit of detection (defined as the human OPG concentration giving an absorbance higher than the mean absorbance of blank* plus three standard deviations: $A_{\text{blank}} + 3 \times SD_{\text{blank}}$) is defined as follows:

- **Analytical Limit of Detection** is calculated from the real osteoprotegerin values in wells and is 0.13 pmol/L.
- **Assay Sensitivity** takes the dilution of samples into consideration and is calculated according to the formula:

$$\text{Assay Sensitivity} = \text{Analytical Limit of Detection} \times \text{sample dilution} = 0.13 \text{ pmol/L} \times 3 = 0.4 \text{ pmol/L}$$

*Dilution Buffer is added into empty wells.

Specificity

Approximately 1% cross-reactivity with recombinant mouse OPG, less than 0.06% with recombinant human CD40, rec. human sTNF RI and sTNF RII.

Precision

Intra assay (Within Run)

Sample (n=10)	Mean (pmol/L)	SD (pmol/L)	CV (%)
Sample 1	6.38	0.13	2.1
Sample 2	5.27	0.18	3.5

Inter assay (Run-to-Run)

Sample (n=10)	Mean (pmol/L)	SD (pmol/L)	CV (%)
Sample 1	6.38	0.27	4.2
Sample 2	5.27	0.32	6.1

Spike Recovery

Serum samples were spiked with different amounts of human OPG and assayed. The results are summarized in the following table:

Sample	Observed (pmol/L)	Expected (pmol/L)	Recovery O/E (%)
1	4.83	-	-
	6.20	6.66	93.1
	11.73	12.05	97.3
	17.68	20.17	87.7
2	5.93	-	-
	7.51	7.75	96.9
	13.81	13.15	105.0
	20.07	21.27	94.4
3	4.39	-	-
	7.39	7.61	97.1
	14.87	15.42	96.4
	16.15	16.97	95.2
4	3.64	-	-
	5.36	5.78	92.7
	9.24	10.99	84.1
	16.81	17.67	95.1

Linearity

Serum samples were diluted with Dilution Buffer and tested for Linearity on Dilution. The following table summarizes the linearity data:

Sample	Dilution	Observed (pmol/L)	Expected (pmol/L)	Recovery O/E (%)
1	-	10.79	-	-
	1:2	5.26	5.40	97.4
	1:4	2.79	2.70	103.3
	1:8	1.47	1.35	108.9
2	-	6.75	-	-
	1:2	3.29	3.38	97.3
	1:4	1.79	1.69	105.9
	1:8	0.96	0.85	112.9
3	-	5.37	-	-
	1:2	2.13	2.69	79.2
	1:4	1.51	1.35	111.9
	1:8	0.79	0.67	117.9
4	-	6.00	-	-
	1:2	3.06	3.00	102.0
	1:4	1.71	1.50	114.0
	1:8	0.65	0.75	86.7

ASSISTANCE

To place an order or for technical assistance, please contact a Quidel Representative at 800-524-6318 or 408-616-4301, Monday through Friday, between 8:00 a.m. and 5:00 p.m., Pacific Time. Orders may also be placed by fax at 408-616-4310.

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

REFERENCES

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GLOSSARY



Instructions for Use on CDROM

REF 8034 – **MICROVUE** OPG EIA Kit
Bone Health



Manufactured for:
QUIDEL[®]
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RESEARCH TO RAPIDS[®]

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