

OSTEOMARK® NTx Urine

An Enzyme-linked Immunosorbent Assay (EIA) for the Measurement of Cross-linked N-telopeptides of Type I Collagen (NTx) in Human Urine

REF 9006

For *in vitro* diagnostic use only

Intended Use of the Osteomark® NTx Urine Assay

The Osteomark® NTx Urine assay is a urinary assay that provides a quantitative measure of the excretion of cross-linked N-telopeptides of type I collagen (NTx) as an indicator of human bone resorption. Elevated levels of urinary NTx indicate elevated human bone resorption. Measurement of NTx is intended for use in:

- Predicting skeletal response (bone mineral density) to hormonal antiresorptive therapy in postmenopausal women
- Therapeutic monitoring of:
 - antiresorptive therapies in postmenopausal women
 - antiresorptive therapies in individuals diagnosed with osteoporosis
 - antiresorptive therapies in individuals diagnosed with Paget's disease of bone
 - estrogen-suppressing therapies
- Identifying the probability for a decrease in bone mineral density after one year in postmenopausal women receiving calcium supplement relative to those treated with hormonal antiresorptive therapy.

The measurement range of the Osteomark® NTx Urine assay is 20 to 3000 nM Bone Collagen Equivalents (BCE).

Summary and Explanation of the Test

Mammalian bone is continuously remodeled through a coupled process of osteoclast mediated bone resorption, followed by osteoblast mediated bone formation. This process is necessary for normal development and maintenance of the skeleton. Abnormalities in this tightly coupled process often result in changes in skeletal mass and shape. The measurement of specific degradation products of bone matrix provides analytical data of the rate of bone metabolism. Approximately 90% of the organic matrix of bone tissue is type I collagen. Type I collagen, a helical protein that is cross-linked at the N-terminal and C-terminal ends of the molecule, forms the basic fabric and tensile strength of bone tissue. The discovery of urinary cross-linked N-telopeptides of type I collagen (NTx) has provided a specific biochemical marker of human bone resorption which can be analyzed by immunoassay. The NTx molecule is specific to bone due to the unique amino acid sequences and orientation of the cross-linked alpha-2 (I) N-telopeptide. Generation of the NTx molecule is mediated by osteoclasts on bone, and is found in the urine as a stable end-product of degradation.

The Osteomark® NTx Urine assay provides a quantitative measure of the excretion of NTx as an indicator of human bone resorption. Elevated levels of urinary NTx indicate elevated bone resorption. Clinical research has demonstrated that elevated bone resorption is the primary cause of age-related bone loss and that low bone mass often results in osteopenia and is the major cause of osteoporosis.^{1,2} Loss of bone mass occurs when bone resorption levels are elevated above bone formation levels. Osteoporotic fractures are reported to be the major source of increased morbidity and mortality in older women.³

A randomized, prospective clinical trial of postmenopausal women treated with either hormone replacement therapy (HRT) plus calcium supplements (500 mg daily) or calcium supplements alone, supported the use of the Osteomark® NTx Urine assay to predict response and monitor the antiresorptive effect of the therapy.⁴

The ability of the Osteomark® NTx Urine assay to monitor the effect of antiresorptive therapy has been demonstrated in a study of patients diagnosed with osteoporosis treated with a bisphosphonate (alendronate sodium, 10 mg).⁵ Results demonstrate that the osteoporotic patient should be managed to an Osteomark® NTx Urine assay value of ≤ 35 nM BCE/mM creatinine or have a $\geq 40\%$ change from baseline after 3 months of treatment.

A study was conducted to determine the ability of the Osteomark® NTx Urine assay to monitor the effect of antiresorptive therapy in patients with Paget's disease of bone. The Osteomark® NTx Urine assay values at baseline and the percent change from baseline after therapy initiation correlated to serum total alkaline phosphatase. The Osteomark® NTx Urine assay provided an earlier assessment than alkaline phosphatase of therapeutic response, defined by normalization of values. Paget's patients treated with antiresorptive therapy should have a $\geq 30\%$ change in the Osteomark® NTx Urine assay from baseline after 3 months of treatment.

Assay Principles

The Osteomark® NTx Urine assay is a competitive-inhibition enzyme-linked immunosorbent assay (ELISA) that utilizes microwells as the solid phase onto which NTx has been adsorbed. NTx in the specimen competes with the solid phase NTx for binding sites of a monoclonal antibody labeled with horseradish peroxidase. The amount of antibody bound to the solid phase is therefore inversely proportional to the amount of NTx in the specimen. Quantitation of the NTx concentration in the specimen is determined spectrophotometrically and calculated from a standard calibration curve. Assay values are corrected for urinary dilution by urinary creatinine analysis and expressed in nanomoles bone

collagen equivalents per liter (nM BCE) per millimole creatinine per liter (mM creatinine).

Kit Components

Supplied Materials Sufficient for 96 wells

	Instructions for Use (1 booklet)	1 booklet
A	Antigen Coated 96-well Plate, 12 1x8-well strips	1 Plate
B	Antibody Conjugate Concentrate	0.4 mL vial
C	Antibody Conjugate Diluent	30 mL bottle
D	30X Wash Concentrate	125 mL bottle
E	Chromogen Reagent	0.9 mL bottle
F	Buffered Substrate	30 mL bottle
G	Stopping Reagent	25 mL bottle
1	1 nM BCE Calibrator	0.4 mL vial
2	30 nM BCE Calibrator	0.4 mL vial
3	100 nM BCE Calibrator	0.4 mL vial
4	300 nM BCE Calibrator	0.4 mL vial
5	1000 nM BCE Calibrator	0.4 mL vial
6	3000 nM BCE Calibrator	0.4 mL vial
I	Level I Urine Control	0.4 mL vial
II	Level II Urine Control	0.4 mL vial
	Plate Sealers	1 pad

Reagent Description

PLATE	Antigen Coated 96-Well Plate , 12 1x8 well strips Purified human NTx antigen adsorbed onto microwell strips.
CONJ	Antibody Conjugate Concentrate , 1 vial Purified murine monoclonal antibody directed against NTx and conjugated to horseradish peroxidase. ProClin™ 300 (0.10%) is included as a preservative. Supplied as a 100x concentrated reagent.
CONJ DIL	Antibody Conjugate Diluent , 1 bottle Buffered reagent with protein stabilizers, into which Antibody Conjugate Concentrate is diluted. ProClin™ 300 (0.05%) included as a preservative.
WASHBUF 30x	30x Wash Concentrate , 1 bottle Ionic detergent solution. Supplied as a 30x concentrate.
CHROMOGEN	Chromogen Reagent , 1 vial 3,3',5,5' – tetramethylbenzidine in dimethyl-sulfoxide. Supplied as a 100x concentrated reagent.
BUFFER	Buffered Substrate , 1 bottle Buffered hydrogen peroxide.
SOLN STOP	Stopping Reagent , 1 bottle 1N sulfuric acid.
CAL xx nM	Assay Calibrators 1, 30, 100, 300, 1000, 3000 nM BCE , 1 vial each Purified NTx antigen is buffered in diluent. ProClin™ 300 (0.05%) included as a preservative.
CONTROL xx	Level I and Level II Urine Controls , 1 vial each Human urine with known NTx concentration. ProClin™ 300 (0.10%) included as a preservative.
PLATE SEALERS	Plastic Plate Sealer , 1 pad Note: Bovine Serum or Bovine Serum Albumin is present in some components.

Storage of Reagents

Reagents must be stored at 2 - 8 °C when not in use. Reagents must be brought to room temperature before use. Do not expose reagents to temperatures greater than 30 °C or less than 2 °C. Diluted wash solution may be stored at room temperature for up to one month.

Materials Required but Not Supplied

- Single and multichannel pipettes capable of delivering 25 µL, 100 µL and 200 µL volumes.
- Disposable pipet tips.
- Disposable plastic containers for reagent mixing and pipetting reservoirs.
- Automated microwell washer.
- Microwell or microstrip spectrophotometric reader. The reader must read at 450nm with a 630 nm reference filter and detect absorbances from 0 to 3.000 optical density units.
- Software capable of calculating a 4-parameter curve fit.
- Deionized water.

Urine Specimen Collection and Storage

- Collect a second void of the morning (spot) urine specimen or a 24 hour urine specimen in an appropriate collection device with a tight fitting lid.
- DO NOT ADD PRESERVATIVE TO URINE SPECIMEN.
- Specimens with visible whole blood contamination or visible hemolysis may interfere with the assay and should be discarded. Collection of a new specimen is recommended.
- Store refrigerated (2 - 8 °C) for up to 72 hours or at room temperature for up to 24 hours. Store frozen (-20 °C or below) for longer term storage. Specimens may undergo three freeze/thaw cycles.
- When monitoring therapy, baseline samples should be collected prior to initiation of therapy. Subsequent specimens for comparison should be collected at the same time of day as the baseline specimen.**

Warnings and Precautions

- For *in vitro* diagnostic use only**
- The Antigen Coated 96-well Plate, Calibrators, and Urine Controls contain human urine and/or antigen processed from human bone tissue. Although each lot of urine and bone has been documented to be non-reactive for HIV 1, HIV 2, HBsAg, HCV and RPR by FDA approved methods, these materials should be handled as potentially infectious and should be disposed of properly.
-  The Stopping Reagent contains 1N sulfuric acid
Signal word: Danger
Hazard statements: H314 - Causes severe skin burns and eye damage, H318 - Causes serious eye damage
-  The Chromogen Reagent contains 3,3',5,5'- tetramethylbenzidine and dimethylsulfoxide. Dimethylsulfoxide is readily absorbed through the skin.
Signal word: Warning
Hazard statements: H315 - Causes skin irritation, H319 - Causes serious eye irritation, H227 - Combustible liquid.
-  ProClin is included as a preservative in most reagents, at concentrations listed in the reagent section.
Signal word: Warning
Hazard statements: H315 - Causes skin irritation, H317 - May cause an allergic skin reaction, H319 - Causes serious eye irritation.
- Well plate, Assay Calibrators, Urine Controls: Contains materials of human origin.
- Urine specimens may contain infectious agents and should be disposed of properly. Decontamination is most effectively accomplished with a 0.5% solution of sodium hypochlorite (1:10 dilution of household bleach) or by autoclaving one hour at 121 °C. Do not autoclave solutions containing sodium hypochlorite. Do not combine sodium hypochlorite solution with acid.
- Never pipette reagents or clinical specimens by mouth.
- Wear protective gloves and clothing when handling specimens and reagents. Wash hands thoroughly after use.
- Do not use reagents beyond their expiration dates.
- Do not mix components from different lots of the Osteomark® NTx Urine assay kits.
- Microwell strips must be kept desiccated. Reseal unused microwell strips in the pouch containing desiccant.
- Do not reuse microwells. Dispose of properly after use.
- Perform the assay procedure in a controlled laboratory environment that adheres to the stated incubation requirements. Avoid extreme environmental conditions during the procedure.

Note: Safety data sheets are available for professional users upon request.

Assay Procedure

Preparatory Steps

- Allow all specimens and reagents to equilibrate to room temperature (18 - 28 °C) for at least one hour before performing the assay.** To facilitate warming, remove reagents from the kit box. Frozen urine specimens may be thawed at 37 °C, in either a water bath or an incubator, then brought to room temperature prior to use in the assay. The Chromogen Reagent contains dimethylsulfoxide, which may solidify when refrigerated but is liquid at room temperature.
- Prepare the working strength wash solution. Dilute 30X Wash Concentrate 1:30 with deionized water (1 part 30X Wash Concentrate to 29 parts deionized water) and mix for a minimum of five (5) minutes. This solution is stable for one (1) month at room temperature.
- Create a plate map indicating location of calibrators, controls and urine specimens. It is recommended that calibrators, controls and urine specimens be run in duplicate microwells. An example of a plate map is provided below for an Osteomark® NTx Urine assay with 4 specimens:

	1	2	3
A	1 nM BCE Calibrator	1000 nM BCE Calibrator	Specimen #1
B	1 nM BCE Calibrator	1000 nM BCE Calibrator	Specimen #1
C	30 nM BCE Calibrator	3000 nM BCE Calibrator	Specimen #2
D	30 nM BCE Calibrator	3000 nM BCE Calibrator	Specimen #2
E	100 nM BCE Calibrator	Level I Urine Control	Specimen #3
F	100 nM BCE Calibrator	Level I Urine Control	Specimen #3
G	300 nM BCE Calibrator	Level II Urine Control	Specimen #4
H	300 nM BCE Calibrator	Level II Urine Control	Specimen #4

- Using a clean disposable plastic container, dilute the Antibody Conjugate Concentrate 1:101 using the Antibody Conjugate Diluent. As a guideline, for each Antigen Coated microwell strip used, dilute 20 µL Antibody Conjugate Concentrate into 2 mL of Antibody Conjugate Diluent. Mix gently by inversion only. **Do not vortex or use a magnetic stir bar.** Avoid foaming. Use the working strength conjugate solution within one hour of preparation. Do not reuse the container.
- Prior to pipetting, gently mix the Calibrators, Controls and urine specimens. Avoid foaming. Allow cloudy or turbid specimens to settle 5 to 10 minutes prior to pipetting. Urine specimens containing particulates may be centrifuged before use.
- Remove the appropriate number of microwell strips from the sealed foil pouch. Place any unused strips back in the pouch, resealing the pouch along the zipper. Do not remove the desiccant pillow from the foil pouch.

Specimen and Antibody Incubation

Once the assay has been started, complete it without interruption.

- Following the plate map generated in Step 3, pipette 25 µL of each Calibrator, Control, or urine specimen into the bottom of the designated microwells. Use a calibrated pipettor and new pipette tips for each Calibrator, Control, or urine specimen.
- Using a multichannel pipettor, deliver 200 µL of the working strength conjugate solution into each microwell. Apply a plate sealer and swirl the plate gently on a flat surface for 5-10 seconds to ensure mixing.
- Incubate the plate at room temperature (18 - 28 °C) for 90 ± 5 minutes.
- Prepare the Chromogen/Buffered Substrate solution during the last 10 minutes of incubation by making a 1:101 dilution of the Chromogen Reagent into the Buffered Substrate. As a guideline, for each Antigen Coated microwell strip used, dilute 20 µL of the Chromogen Reagent into 2 mL of the Buffered Substrate. Pipette the Buffered Substrate into a clean plastic disposable container. Thoroughly mix the Chromogen Reagent prior to pipetting. Add the Chromogen Reagent to the Buffered Substrate Reagent and invert gently to mix. **Do not vortex, shake vigorously or use a magnetic stir bar to mix. Use the Chromogen/Buffered Substrate solution within 30 minutes of preparation.** The Chromogen/Buffered Substrate solution should be colorless when mixed. A blue color indicates that the reagent has been contaminated and must be discarded. **Do not reuse the Chromogen/Buffered Substrate solution container.**
- At the end of the incubation period, carefully remove and discard the plate sealer. Wash the plate five (5) times with the working strength wash solution using an automated plate washer. The automated washer must dispense at least 350 µL of the working strength wash solution per well. Between wash cycles, wells should be filled with working strength wash solution. When wash procedure is complete, grasp the plate frame at the center of each side and invert, blotting on an absorbent paper towel. Immediately add the prepared Chromogen/Buffered Substrate solution as described below.

Color Development and Measurement

- Pipet 200 µL of the Chromogen/Buffered Substrate solution prepared in step 10 into each well using a multichannel pipettor. Cover the plate with a new plate sealer.
- Incubate at room temperature for 15 ± 1 minutes. A blue color will develop in wells containing bound antibody-horseradish peroxidase conjugate.
- Following incubation, carefully remove and discard the plate sealer. Using a multichannel pipettor, add 100 µL of Stopping Reagent to each well in the same order as addition of the Chromogen/Buffered Substrate Reagent. Wells which have developed a blue color will now turn yellow.
- Swirl the plate gently on a flat surface for 5 -10 seconds to ensure mixing. Allow the plate to sit at room temperature for 5 minutes before reading absorbance values.
- Within 30 minutes of adding the Stopping Reagent, read the absorbance of the Calibrators, Controls, and urine specimens. Use a microwell plate reader at 450 nm with a reference filter of 630 nm. The reader must have a maximum optical density reading of ≥3.000.

interfere with assay performance. Human albumin, bilirubin, glucose, and vitamin C did not interfere with assay performance. Urine specimens obviously contaminated with whole blood or that have visible hemolysis may interfere with assay performance. These specimens should be discarded, and a new urine specimen collected.

Expected Values

A multi-center, cross-sectional study was conducted to determine the reference range for normal premenopausal women (mean age 36 years, range 25-49) and men (mean age 56 years, range 31-87).

Table 1:

Expected Osteomark® NTx Urine Assay Values for Men and Premenopausal Women Range

	Mean*	Std Dev	Range (mean ± std dev)*	N
Women	35	15	5-65	258
Men	33	15	3-63	206

* expressed as nM BCE/mM creatinine

When the premenopausal women expected value range is log-transformed, the range is 14-74 nM BCE/mM creatinine. The log-transformed reference range for men is 13-78 nM BCE/mM creatinine.

The expected short- and long-term intra-subject variability was determined from prospective studies conducted in healthy postmenopausal women (n=276, 268) and men greater than 30 years of age (n=36, 35). Short-term variability was determined from sequential urine specimens collected over 3-4 days and long-term variability was determined from 2-3 monthly collections. Short-term variability was 15% for women and 18% for men. Long-term variability was 18% for women and 19% for men.

Performance Characteristics

Reproducibility and Precision

Intra-assay variability was assessed using eight urine specimens tested in replicates of 10 by each of four operators. Results are provided below:

Sample	Mean (nM BCE)	CV (%)
A	26	19
B	111	8
C	172	8
D	417	5
E	694	5
F	1113	5
G	1768	6
H	2640	5

Inter-assay variability was assessed using three urine specimens tested in duplicate by one operator over 20 separate assay runs. Results are provided below:

Sample	Mean (nM BCE)	CV (%)
A	79	5
B	412	3
C	1167	4

Total assay precision was evaluated by testing the Level I Urine Control and the Level II Urine Control at three clinical laboratory sites over a 30 day period. The Level I Urine Control had a mean value of 439 nM BCE, with a 10% CV. The Level II Urine Control had a mean value of 1537 nM BCE, with a 7% CV.

Antigen Recovery

Antigen recovery was evaluated by adding known amounts of NTx to each of three urine specimens of known NTx concentration. Recovery represented the observed assay value of the "spiked" specimens, calculated as a percent of the expected urine value (baseline urine value plus added antigen (NTx) value). The results demonstrated an average antigen recovery of 105% across the assay range.

Dilutional Linearity

Dilutional linearity was evaluated by performing serial dilutions of four urine specimens with high nM BCE values into a urine specimen with a 200-500 nM BCE value. Results demonstrated correlation coefficients of $r = 0.999$ to $r = 1.000$ across the assay range.

Clinical Studies

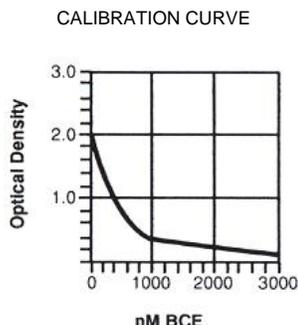
Use of the Osteomark® NTx Urine Assay in Postmenopausal Women

A clinical trial was conducted to determine the ability of the Osteomark® NTx Urine assay to monitor the effect of hormone replacement therapy (HRT) on bone resorption in early postmenopausal women, and to predict response to HRT. Results support the clinical utility of the Osteomark® NTx Urine assay to monitor hormonal antiresorptive therapy in early postmenopausal women and to

Analysis of Results

- Determine concentration values (nM BCE) of Controls and urine specimens using a 4-parameter curve fitting equation.

EXAMPLE:



- Assay results are valid if the following criteria are met:
 - The mean absorbance value of the 1 nM BCE Calibrator must be ≥ 1.500 .
 - The span of the calibrator curve (difference between absorbance values of the 1 nM BCE and 3000 nM BCE Calibrators) must be ≥ 1.300 .

- The recommended coefficient of variation (% CV) between urine specimen concentration value (nM BCE) duplicates is $\leq 20\%$. Specimens with $>20\%$ CV should be rerun.
- The lower limit of detection is 20 nM BCE (assay value, does not include creatinine correction).
- Urine specimens that exceed 3000 nM BCE may be diluted 1:5 in a urine specimen or pool of urine known to be within the range of 200-500 nM BCE, and retested. When using urine as a diluent, the nM BCE of the urine diluent should be confirmed by testing it as a specimen in the same plate as the diluted unknown specimen. The dilution factor and background (the diluent nM BCE) should be incorporated into the final calculation.

Example: 1040 nM BCE assay value derived from a 1:5 dilution of a 4000 nM BCE specimen using a urine diluent of known Osteomark® NTx Urine assay value (300 nM BCE)

$$1040 \text{ nM BCE} - (0.8 \times 300 \text{ nM BCE}) = 800 \text{ nM BCE}$$

$$800 \text{ nM BCE} \times 5 \text{ (dilution factor)} = 4000 \text{ nM BCE}$$

Note: 1:5 dilutions represent 80% diluent (0.8), 20% specimen contribution.

- Report the concentration values for urine specimens as nM BCE/mM creatinine, as shown in the following example:

$$\begin{aligned} \text{Assay value} &= 360 \text{ nM BCE} \\ \text{Urinary creatinine} &= \frac{60 \text{ mg/dL creatinine}}{11.3^*} \\ &= 5.3 \text{ mM creatinine} \\ \frac{360 \text{ nM BCE}}{5.3 \text{ mM creatinine}} &= 68 \text{ nM BCE/mM creatinine} \end{aligned}$$

*Note: Conversion factor used to convert mg creatinine per dL to millimole creatinine per liter.

- These Urine Control ranges have been established by the manufacturer. It is recommended that each laboratory establish its own control ranges.

Limitations of the Procedure

While the Osteomark® NTx Urine assay is used as an indicator of bone resorption, the use of this test has not been established to predict development of osteoporosis or future fracture risk. Use of this test has not been established in primary hyperparathyroidism or hyperthyroidism. When using the Osteomark® NTx Urine assay to monitor therapy, results may be confounded in patients afflicted with clinical conditions known to affect bone resorption, e.g., metastases to bone. While an Osteomark® NTx Urine assay value provides a measure of the level of bone resorption, a single Osteomark® NTx Urine assay value cannot provide the rate of bone resorption as reported results do not contain a measure of time. The Osteomark® NTx Urine assay results should be interpreted in conjunction with clinical findings and other diagnostic results.

Interfering Substances

Various urine components and microorganisms were evaluated for an interfering effect in the Osteomark® NTx Urine assay. The organisms tested, *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), and *C. albicans* (ATCC 14053), did not

predict changes in bone mineral density (BMD) measured by dual energy x-ray absorptiometry (DEXA) in response to HRT, thereby identifying who will receive the greatest benefit from such therapy, and to identify those at risk for bone loss as measured by BMD.⁴ Examination of the contrast between treated and control groups provides information regarding risk of BMD loss (Table 2): In the lowest NTx quartile (≤ 38 nM BCE/mM creatinine), there was no statistically significant difference between the HRT and control groups in the likelihood of bone loss over 1 year. A high baseline NTx (>67 nM BCE/mM creatinine) indicated a 17.3 times higher risk of BMD loss if not treated with HRT.

Baseline NTx* (nM BCE/mM creatinine)	Relative Risk	95% C.I
18-38	1.4	0.8 – 2.5
38-51	2.5	1.0 – 6.1
51-67	3.8	1.6 – 9.1
67-188	17.3	2.5 – 118.5

*N = 54, 53, 57, and 55 for each quartile respectively

Use of the Osteomark® NTx Urine Assay in Patients Diagnosed with Osteoporosis

A study was conducted to establish the safety and efficacy of a new amino-bisphosphonate (alendronate) in the treatment of osteoporosis.⁵ The following data support the clinical utility of the Osteomark® NTx Urine assay to monitor levels of bone resorption in osteoporotic women treated with antiresorptive therapy (alendronate 10 mg).

- Three months after initiating treatment 80% (71/89) of the subjects had an Osteomark® NTx Urine assay value ≤ 35 nM BCE/mM creatinine and 87% (76/87) had a $\geq 40\%$ decrease from baseline.
- After 1 year of therapy, 90% (77/86) of the subjects had an Osteomark® NTx Urine assay value ≤ 35 nM BCE/mM creatinine and 92% (77/84) of the subjects had a $\geq 40\%$ decrease from baseline.
- Therefore, the Osteomark® NTx Urine assay values should be monitored in osteoporotic women treated with antiresorptive therapy to a value of ≤ 35 nM BCE/mM creatinine, or a $\geq 40\%$ decrease from baseline after 3 months.

Use of the Osteomark® NTx Urine Assay in Patients Diagnosed with Paget's Disease of Bone

A study was conducted to determine the ability of the Osteomark® NTx Urine assay to monitor the effect of bisphosphonate therapy on bone resorption in patients diagnosed with Paget's disease of bone. The following data support the clinical utility of the Osteomark® NTx Urine assay to monitor the effect of bisphosphonate therapy on bone resorption levels in patients diagnosed with Paget's disease of bone.

Visit	Alendronate	Etidronate	Pamidronate
Month 1*	- 48%	- 39%	- 71%
Month 3**	- 77%	- 58%	- 67%
Month 6***	- 87%	- 72%	- 71%

*N = 22, 17, and 20 respectively

**N = 28, 23, and 20 respectively

***N = 27, 22, and 20 respectively

*The Osteomark® NTx Urine assay values should be monitored in Paget's disease of bone patients treated with antiresorptive therapy to a $\geq 30\%$ change or into the normal range.

Table 4.

Percent Responders as Defined by Normalization of Marker (Osteomark® 5-65 nM BCE/mM creatinine, total alkaline phosphatase 39-115 IU/L; number of patients with normalized marker value/total number of patients)

Visit*	Osteomark® Responders	Total Alkaline Phosphatase Responders
Month 1	19%	2%
Month 3	34%	28%
Month 6	42%	42%

*N = 59, 71, and 69 respectively

- A decrease in total alkaline phosphatase into its normal range is used as an indicator of therapeutic response. The Osteomark® NTx Urine assay values also measure the response and provide an earlier assessment than total alkaline phosphatase.

Use of the Osteomark® NTx Urine assay to Monitor Estrogen Suppressing Therapy

A clinical trial was conducted to determine the ability of the Osteomark® NTx Urine assay to monitor the effect of estrogen suppressing therapy on bone resorption in premenopausal women.⁶ The results support the clinical utility of the Osteomark® NTx Urine assay to monitor the effect of estrogen suppressing therapy on bone resorption levels.

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Osteomark® NTx Urine Assay Quick Reference

1. Thoroughly read the Assay Procedure before you begin.
2. **Bring kit components and specimens to room temperature.**
3. Dilute the Antibody Conjugate Concentrate into the Antibody Conjugate Diluent, using a 1:101 ratio. Mix gently by inversion only. Use the working strength conjugate solution within one hour of preparation.
4. Pipette 25 μ L samples of each Calibrator, Control, and urine specimen into designated microwells.
5. Pipette 200 μ L of the working strength conjugate solution into each microwell. Gently swirl to mix. Incubate the plate at room temperature for 90 ± 5 minutes.
6. Prepare Chromogen Reagent/Buffered Substrate solution during the last 10 minutes of incubation. Dilute the Chromogen Reagent into the Buffered Substrate using a 1:101 ratio. Mix gently BY INVERSION ONLY. **Do not vortex, shake vigorously, or mix with a magnetic stir bar. Use the Chromogen/Buffered Substrate within 30 minutes of preparation.**
7. Wash microwells five (5) times with the working strength wash solution at 350 μ L per well, and blot on absorbent paper towel.
8. Add 200 μ L of Chromogen/Buffered Substrate to each microwell, and incubate at room temperature for 15 ± 1 minutes.
9. Add 100 μ L of Stopping Reagent to each microwell. Gently swirl the plate to mix.
10. Incubate at room temperature for 5 minutes and read the absorbance of each microwell at 450 nm - 630 nm. Calculate the results using a 4-parameter curve fitting equation.

Ordering and Contact Information:

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Technical Support:

Advice Line

Further information can be obtained from your distributor, or by contacting Alere Technical Support on:

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Hazard pictograms. See precautions.	CE Mark	Authorized Representative in the European Community	Manufacturer	Catalog number



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IN9006E Rev.6 2017/03

RX7032E