Version 14-04/24

User's Manual

Human NTx Serum ELISA Kit

REF **DEIA-S10026**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

A Serum NTx level is used to aid in predicting skeletal response (bone mineral density) to antiresorptive therapy and in monitoring bone resorption changes following initiation of antiresorptive therapy. Prior to initiating antiresorptive therapy, a serum NTx level is used to determine the probability for a decrease in bone mineral density (BMD) after one year in postmenopausal women treated with hormonal antiresorptive therapy relative to those treated with calcium supplementation.

The measurement range of NTx Serum is 3.2 to 40.0 nM Bone Collagen Equivalents (BCE).

Principles of Testing

NTx Serum is a competitive-inhibition enzyme-linked immunosorbent assay (ELISA/EIA) for quantitative determination of NTx in human serum.

NTx epitope is adsorbed onto a 96-well microplate. Diluted samples are added to the microplate wells, followed by a horseradish peroxidase labeled monoclonal antibody. NTx in the patient sample competes with the NTx epitope in the microplate well for antibody binding sites. Following a wash step, the amount of labeled antibody bound is measured by colorimetric generation of a peroxide substrate. Absorbance is determined spectrophotometrically and NTx concentration calculated using a standard calibration curve. Assay values are reported in nanomoles Bone Collagen Equivalents per liter (nM BCE).

Reagents And Materials Provided

Supplied materials sufficient for 96 wells:

	Instructions for Use	1 booklet
A	Antigen Coated 96-well Plate, 12 1x8-well strips	1 plate
B	Specimen Diluent	40 mL bottle
C	Antibody Conjugate Concentrate	0.4 mL vial
D	Antibody Conjugate Diluent	25 mL bottle
E	Chromogen Reagent	0.9 mL bottle
F	Buffered Substrate	30 mL bottle
G	Stopping Reagent	25 mL bottle
H	30X Wash Concentrate	125 mL bottle
0	0 nM BCE Calibrator	20 mL vial
5	5 nM BCE Calibrator	0.4 mL vial
10	10 nM BCE Calibrator	0.4 mL vial
20	20 nM BCE Calibrator	0.4 mL vial
40	40 nM BCE Calibrator	0.4 mL vial
I	Level I Serum Control	0.4 mL vial
II	Level II Serum Control	0.4 mL vial
	Plate Sealers	1 pad

Materials Required But Not Supplied

1. Precision single and multichannel pipettes.
2. Disposable pipette tips. (New pipette tips must be used for each addition of different specimens or reagents during the assay procedure).
3. Microtubes or equivalent for preparing dilutions.
4. Disposable plastic containers for preparing working conjugate and chromogen solutions.

5. Reagent reservoirs.
6. Automated microwell washer.
7. Microwell or microstrip plate reader with 450nm and 630nm filters.
8. Software capable of computing results using a 4-parameter logistic curve-fitting equation.
9. Deionized water.

Storage

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 25°C. Diluted wash solution may be stored at room temperature for up to one month.

Specimen Collection And Preparation

Human serum collected by standard venipuncture technique is used in the NTx Serum. The use of plasma samples has not been established. Allow blood to fully clot and remove the serum from the red blood cells promptly. Specimens collected in serum separation tubes should be removed from the gel. Store serum samples refrigerated (2 - 8°C) for up to 24 hours, or store frozen (-20°C or below) for longer term storage. Specimens may undergo three freeze/thaw cycles.

For monitoring therapy, baseline samples should be collected just prior to or on the day of therapy initiation. Subsequent specimens for comparison should be collected at approximately the same time of day as the baseline specimen.

Assay Procedure

Preparatory Steps

1. Allow all specimens and kit components to equilibrate to room temperature (20 - 25°C). Mix all reagents thoroughly. Avoid foaming.
2. Prepare working strength wash solution. Dilute 30X Wash Concentrate 1: 30 with deionized water (1 part 30X Wash Concentrate with 29 parts deionized water; example dilution would be 30 mL Wash Concentrate plus 870 mL deionized water) and mix for a minimum of five (5) minutes. The diluted wash solution is stable for one (1) month at room temperature.
3. Plan the plate configuration, and create a plate map. It is recommended that each calibrator and control be run in duplicate. An example for 10 specimens is below:

	1	2	3
A	0 Calibrator	40 Calibrator	Specimen #3
B	0 Calibrator	40 Calibrator	Specimen #4
C	5 Calibrator	Level I Cont.	Specimen #5
D	5 Calibrator	Level I Cont.	Specimen #6
E	10 Calibrator	Level II Cont.	Specimen #7
F	10 Calibrator	Level II Cont.	Specimen #8
G	20 Calibrator	Specimen #1	Specimen #9
H	20 Calibrator	Specimen #2	Specimen #10

4. Prepare working strength conjugate solution. Using a clean disposable plastic container, dilute the Antibody Conjugate Concentrate to a 1: 101 ratio using Antibody Conjugate Diluent. Mix gently by inversion only. Do

not vortex or use a magnetic stir bar. Avoid foaming. Do not reuse the container. Use the following table as a guideline for reagent preparation.

Total Number of Strips	Conjugate Concentrate (µL)	Conjugate Diluent (mL)
3-4	40	4
5-8	80	8
9-12	120	12

5. Thoroughly mix the Calibrators, Controls and specimens.
6. Prepare 1: 5 dilutions of all Calibrators, Controls and specimens with Specimen Diluent in microtubes, or equivalent (1 part sample and 4 parts Specimen Diluent). A minimum volume of 200 µL diluted sample is required for each sample. (e.g. 50 µL sample + 200 µL diluent). Vortex the diluted samples to mix thoroughly, avoid foaming.
7. 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

8. Pipette 100 µL of each diluted Calibrator, Control or sample into the microplate according to the plate configuration. It is recommended that calibrators and controls be run in duplicate. Use a calibrated pipettor and a new pipette tip for each Calibrator, Control, and sample. Immediately proceed to step 9.
9. Using a multichannel pipette, deliver 100 µL of working strength conjugate solution into each microwell. Apply a plate sealer and gently swirl the plate on a flat surface for 15-20 seconds to ensure mixing.
10. Incubate the plate at room temperature (20-25°C) for 90 ± 5 minutes.
11. Prepare Chromogen Reagent/Buffered Substrate solution during the last 5 minutes of incubation. Dilute Chromogen Reagent into Buffered Substrate using a 1: 101 ratio. Use a clean, disposable, plastic container. Do not re-use disposable container. Mix well by inversion only. Do not vortex, shake vigorously or use a magnetic stir bar to mix. (This solution should be colorless when mixed. A blue color indicates that the reagent may be contaminated and should be discarded.) As a guideline, prepare 2 mL of solution (20 µL Chromogen Reagent into 2 mL Buffered Substrate) per strip assayed.
12. At the end of the incubation period, carefully remove and discard the plate sealer. Wash microwells five (5) times with the working strength wash solution using an automated plate washer. Use a minimum wash volume of 350 µL per well per wash cycle. Blot on absorbent paper after the final wash. (Too few or too many washes may cause inaccurate results.) Immediately proceed to step 13. Do not allow strips to dry.

Color Development and Measurement

13. Using a multichannel pipettor, add 200 µL diluted Chromogen Reagent/Buffered Substrate to each microwell. Apply a new plate sealer.
14. Incubate at room temperature (20-25°C) for 30 ± 2 minutes. A blue color will develop in wells containing bound antibody-horseradish peroxidase conjugate.
15. At the end of the incubation, carefully remove and discard the plate sealer.
16. Using the multichannel pipettor, add 100 µL of Stopping Reagent to each well. Wells that have developed a blue color will turn yellow. Swirl the plate gently on a flat surface for 15-20 seconds to ensure mixing. Allow the plate to sit at room temperature (20-25°C) for 5 minutes before reading absorbance values.
17. Within 30 minutes of adding the Stopping Reagent, read the absorbance of the Calibrators, Controls, and specimens using a microwell plate reader (read at 450 nm with a 630 nm reference filter).

Evaluation

A multi-center, cross-sectional study was conducted at five regional sites to determine the reference range for normal premenopausal women (mean age 36 years, range 25-49). The male reference range was determined from a multi-center, cross-sectional study conducted at three regional sites (mean age 51 years, range 31-80).

	Mean*	Std Dev	Range (mean ± 2 Std Dev)	N
Women	12.6	3.2	6.2 – 19.0	257
Men	14.8	4.7	5.4 – 24.2	176

When the expected value range for premenopausal women is log-transformed, the range is 7.7 - 19.3 nM BCE. The log-transformed male range is 8.1 - 24.8 nM BCE. These ranges are provided as guidelines only. Each laboratory should establish their own reference ranges.

A study was conducted to determine the intra-subject variability of serum NTx in postmenopausal women. Subjects provided blood specimens for three consecutive days to assess short-term variability, and for two consecutive months to assess long-term variability. The mean % CV in the short-term specimen set (n=271) was 7.3%. The mean % CV in the long-term specimen set (n=261) was 8.7%.

Intra-subject variability in men was assessed in a subset of the above male reference range study population. The short-term (4 days) intra-subject variability (n=32) was 9.1%, and the long-term (3 months) intra-subject variability (n=27) was 9.5%.

Precision

Total assay precisionwas evaluated by testing the Level I Serum Control (9.4 nM BCE) and the Level II Serum Control (30.0 nM BCE) at four clinical laboratories.

The estimate for the total precision% CV for the Level I Serum Control was 13.99%, and for the Level II Serum Control was 11.92%.

Linearity

Dilutional linearitywas evaluated by performing serial dilutions of five serum specimens with high nM BCE values into a serum specimen with a known low nM BCE value. Percent linearity was determined as the measured value divided by the expected value multiplied by 100. Results demonstrated an average recovery of linear diluted samples of 98%.

Recovery

Antigen Recovery was evaluated by adding known amounts of NTx to each of nine serum specimens of known NTx concentration. Recovery represented the observed assay value of the "spiked" specimens, calculated as a percent of the expected serum value. Results demonstrated an antigen recovery of 94 - 105% across the assay range.

Reproducibility

Intra-assay variabilitywas determined by testing four human serum specimens with BCE values distributed

throughout the calibration range of the assay and following NCCLS Precision Performance Guideline EP5-T2. From these test results the NTx Serum intra-assay variability is established as 4.6%.

Inter-assay variabilitywas determined by testing eight human serum specimens with BCE values distributed throughout the calibration range of the assay. From these test results the NTx Serum inter-assay variability is established as 6.9%.

Interferences

Various serum components were evaluated for an interfering effect on NTx Serum. These components, including total and direct bilirubin, glucose, cholesterol, triglycerides, total protein, albumin and hemoglobin, were tested at levels elevated from physiological norm and did not interfere with assay performance.

Precautions

1. Do not interchange NTx Serum values with NTx Urine values, especially when monitoring therapy.
2. The calibrators and controls contain processed antigen from human bone tissue or human serum. Although each lot 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.
3. Chromogen Reagent contains 3, 3', 5, 5'- tetramethylbenzidine (TMB) and dimethylsulfoxide (DMSO). DMSO is readily absorbed through the skin. If exposed, flush area with water for 15 minutes. If eyes are exposed, get immediate medical attention. TMB is a suspected carcinogen.
4. Serum 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.
5. Never pipette reagents or clinical specimens by mouth.
6. Stopping Reagent contains 1N sulfuric acid. Avoid contact with skin and eyes. If exposed, immediately flush area with water for 15 minutes. If eyes are exposed, get immediate medical attention.
7. Do not use reagents beyond their expiration dates.
8. Do not mix components from other lots of NTx Serum.
9. Microwell strips must be stored desiccated. Do not remove the desiccant pillow from the foil pouch, and reseal any unused strips in the pouch with the desiccant pillow.
10. Do not re-use microwells. Dispose of properly after use.
11. Perform the assay procedure in a controlled laboratory environment that adheres to the stated incubation requirements. Avoid extreme environmental conditions during the procedure.

Limitations

While NTx Serum is used as an indicator of bone resorption, 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, hyperthyroidism, or Paget's disease of bone. When using NTx Serum to monitor therapy, results may be confounded in patients afflicted with other clinical conditions known to affect

bone resorption, e.g. metastases to bone. While an NTx Serum value provides a measure of the level of bone resorption, a single NTx Serum value cannot provide the rate of bone resorption as reported results do not contain a measure of time. NTx Serum results should be interpreted in conjunction with clinical findings and other diagnostic results.

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