INSTRUCTIONS FOR USE

EN

NTx Serum

REF NTx9021 | IVD | Rx Only | Σ 96

INTENDED USE

The NTx Serum is an immunoassay that provides a quantitative measurement of cross-linked N-telopeptides of type I collagen (NTx) in human serum. 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. This test is for *in-vitro* diagnostic use only.

The measurement range of the NTx Serum is 3.2 to 40 nM Bone Collagen Equivalents (BCE).

SIGNIFICANCE AND BACKGROUND

Bone is a dynamic and evolving tissue. Bone tissue undergoes constant bone turnover which involves the destruction of bone (bone resorption) followed by the construction of new bone (bone formation) (1). In mammals, this continuous remodeling of bone is accomplished through a coupled process of osteoclast mediated bone resorption, followed by osteoblast mediated bone formation (2). This process is necessary for normal development and maintenance of the skeleton. When balanced, resorption and formation result in healthy bone tissue. If there should be an abnormal imbalance in these two processes, this may result in changes in skeletal mass and shape.

There are many documented markers of bone resorption. Most fall into one of four categories: collagen degradation products, non-collagenous proteins, osteoclastic enzymes, and osteocyte activity markers (1). NTx falls into the collagen degradation product category. Approximately 90% of the organic matrix of bone tissue is type I collagen. NTx are generated from the amino terminus of the type 1 collagen by cleavage of the N-terminal region by cathepsin K during the resorption phase of bone turnover (1).

Bone turnover markers such as NTx have clinical utility in the comprehensive evaluation of osteoporosis. Elevated concentrations of NTx mean elevated levels of bone resorption. When interpreted with caution and with a good understanding of its natural variability, measurement of NTx may provide information that supplements osteoporosis management and provides useful clinical information about the conditions that alter bone turnover (3).

PRINCIPLE OF THE ASSAY

The NTx Serum assay 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).

TEST SYSTEM COMPONENTS

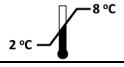
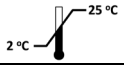
	Kit Component	Quantity (Σ 96)	Description	
A	PLATE	1 Plate	Antigen coated 96-microwell plate. 12 x 8-well strips coated with synthetic NTx antigen.	
B	DILSPE	40 mL bottle	Specimen Diluent. Buffered reagent into which calibrators, controls and specimens are diluted. ProClin™ 300 (0.05%) included as a preservative.	
C	CONJ	0.4 mL vial	Antibody Conjugate Concentrate. Solution containing purified mSerum monoclonal antibody directed against NTx conjugated to horse radish peroxidase and Proclin™ 300 (0.1%). Supplied as a 100X concentrated conjugate.	
D	CONJ DIL	25 mL bottle	Antibody Conjugate Diluent. Buffered reagent with protein stabilizers, into which Antibody Conjugate Concentrate is diluted. ProClin™ 300 (0.05%) included as a preservative.	
E	CHROMOGEN	0.9 mL bottle	Chromogen Reagent. One vial containing a solution of 3,3′,5,5′ – tetramethylbenzidine in dimethyl-sulfoxide. Supplied as a 100x concentrate.	
F	BUFFER	30 mL bottle	Buffered Substrate. One bottle containing a buffered hydrogen peroxide solution.	
G	SOLN STOP	25 mL bottle	Stopping Reagent. One bottle containing a solution of 1N sulfuric acid.	
H	WASHBUF 30X	125 mL bottle	30X Wash Buffer Solution. One bottle containing a 30X ionic detergent solution.	
0	CAL 0nM	20 mL vial	0 nM BCE Calibrator	Various amounts of purified NTx antigen in stabilized protein diluent. Contains ProClin™ 300 (0.05%) as a preservative.
5	CAL 5nM	0.4 mL vial	5 nM BCE Calibrator	
10	CAL 10nM	0.4 mL vial	10 nM BCE Calibrator	
20	CAL 20nM	0.4 mL vial	20 nM BCE Calibrator	
40	CAL 40nM	0.4 mL vial	40 nM BCE Calibrator	
I	CONTROL I	0.4 mL vial	Level I Serum Control	Human serum material with known NTx concentration. ProClin™ 300 (0.10%) included as a preservative.
II	CONTROL II	0.4 mL vial	Level II Serum Control	
	Plate Sealers	1 Pad	Plate Sealers	

NOTE: Note: Bovine Serum or Bovine Serum Albumin is present in some components

MATERIALS REQUIRED BUT NOT PROVIDED

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

STORAGE CONDITIONS

	When not in use, the kit should be stored at 2 – 8 °C. Reagents must be allowed to reach room temperature before use. Do not expose the reagents to temperatures greater than 30 °C or less than 2 °C.
	Diluted (1X) wash solution may be stored at room temperature for up to one month.

PRECAUTIONS

1. **For *in vitro* diagnostic use only.**
2. The Calibrators and Serum Controls contain process antigen from human bone tissue and/or human sera. 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. The Stopping Reagent contains 1N sulfuric acid. Danger: Causes severe skin burns and eye damage.
4. The Chromogen Reagent contains 3,3',5,5'- tetramethylbenzidine and dimethylsulfoxide. Warning - Dimethylsulfoxide is readily absorbed through the skin. May cause skin irritation or serious eye irritation.
5. ProClin is included as a preservative in most reagents, at concentrations listed in the reagent section. May cause skin irritation or serious eye irritation.
6. The Serum Controls: Contain materials of human origin.
7. 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.
8. Never pipette reagents or clinical specimens by mouth.
9. Wear protective gloves and clothing when handling specimens and reagents. Wash hands thoroughly after use.
10. Do not use reagents beyond their expiration dates.
11. Do not mix components from different lots of the NTx Serum assay kits.
12. Microwell strips must be kept desiccated. Reseal unused microwell strips in the pouch containing desiccant.
13. Do not reuse microwells. Dispose of properly after use.
14. 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.

SPECIMEN COLLECTION AND STORAGE

1. Human serum collected by standard venipuncture technique is used in the NTx Serum assay. 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.
2. 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 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 serum 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.
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. Create a plate map indicating location of calibrators, controls and serum specimens. It is recommended that calibrators and Controls be run in duplicate microwells. An example of a plate map is provided below for an NTx Serum assay with 10 patient serum samples:

	1	2	3
A	0 nM BCE Calibrator	40 nM BCE Calibrator	Specimen #3
B	0 nM BCE Calibrator	40 nM BCE Calibrator	Specimen #4
C	5 nM BCE Calibrator	Level I Serum Control	Specimen #5
D	5 nM BCE Calibrator	Level I Serum Control	Specimen #6
E	10 nM BCE Calibrator	Level II Serum Control	Specimen #7
F	10 nM BCE Calibrator	Level II Serum Control	Specimen #8
G	20 nM BCE Calibrator	Specimen #1	Specimen #9
H	20 nM BCE 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 Assay Strips	Conjugate Concentrate (uL)	Conjugate Diluent (mL)
3 to 4	40	4
5 to 8	80	8
9 to 12	120	12
NOTE: Use the diluted conjugate solution within one hour of preparation.		

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

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

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).

ANALYSIS OF RESULTS

1. The NTx Serum assay values are expressed in nanomoles BCE/ L (nM BCE).
2. Determine the concentration values (nM BCE) of Controls and specimens from the calibration curve. The most accurate results are obtained using a 4-parameter logistic curve fitting equation. [NOTE: Some 4-parameter logistic curve fitting equation software packages do not accept a calibrator value of 0, requiring entry of a nominal concentration (such as 0.001) for the 0 nM BCE calibrator.]
3. Assay results are valid if the following criteria are met:
    a. mean absorbance of the 0 nM BCE Calibrator is greater than or equal to 1.300.
    b. the span of the calibrator curve (absorbance difference between 0 nM BCE Calibrator and the 40 nM BCE Calibrator) is greater than or equal to 0.900.
4. If specimens are run in duplicate, the recommended coefficient of variation (% CV) between concentration value (nM BCE) duplicates is ≤20%. Those with >20% CV should be rerun.
5. Patient specimens giving absorbance values below the 40 nM BCE calibrator should be diluted 1:2 in the 0 nM BCE Calibrator (1 part specimen and 1 part 0 nM BCE Calibrator) before diluting 1:5 in Specimen Diluent, and retested. Calculate final result by multiplying the concentration determined from the diluted sample by a factor of 2.
6. These Serum Control ranges have been established by the manufacturer. It is recommended that each laboratory establish its own ranges.

LIMITATIONS OF THE PROCEDURE

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

PERFORMANCE CHARACTERISTICS

Expected Values

Serum NTx concentrations are dependent upon multiple factors. Using the prospectively collected specimens used for the comparative study outlined below, we can report the NTx values for these groups.

Category	n =	Average NTx (nM BCE)	Min NTx (nM BCE)	Max NTx (nM BCE)	Median NTx (nM BCE)
Males, < 25 YOA	25	20	11	42	18
Males, 26-50 YOA	25	13	7	36.7	10.7
Males, > 50 YOA	25	11	5	24	10
Females, 18-35 YOA	25	14	8	28	13
Females, > 50 YOA	50	12	4	32	11

Intra and Inter-Assay Precision

Intra and Inter-Assay precision were evaluated on one lot of the NTx Serum. Briefly, four patient samples were identified that spanned the reportable range of the assay. These four specimens were tested in quadruplicate on each day. The assay was repeated on five days resulting in 20 replicates for each specimen. The results of the Intra-Assay and Inter-Assay precision study are depicted in the table below:

		Intra-Assay Precision					Inter-Assay Precision
		Day1	Day2	Day3	Day4	Day5	All days
Sample 1	Mean	26.7	30.8	24.8	27.1	29.4	27.7
	Standard Deviation	3.04	1.31	1.58	0.62	1.16	2.66
	Percent CV	11.4%	4.2%	6.4%	2.3%	3.9%	9.6%
Sample 2	Mean	17.9	19.6	15.9	15.4	17.6	17.3
	Standard Deviation	0.72	0.22	0.46	0.84	1.52	1.72
	Percent CV	4.0%	1.1%	2.9%	5.5%	8.6%	10.0%
Sample 3	Mean	12.3	15.0	11.0	11.2	12.4	12.3
	Standard Deviation	0.85	0.99	0.29	0.54	1.55	1.68
	Percent CV	6.9%	6.7%	2.6%	4.8%	12.6%	13.6%
Sample 4	Mean	6.6	8.0	6.4	6.5	8.5	7.2
	Standard Deviation	0.99	1.56	0.36	0.73	1.20	1.28
	Percent CV	15.1%	19.6%	5.6%	11.2%	14.2%	17.9%

Lot-to-Lot Precision

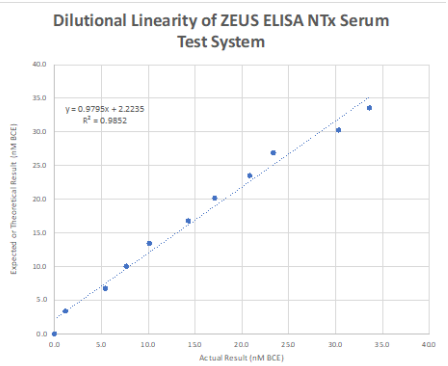
Four samples were chosen that spanned the reportable range of the assay. These samples were tested in 10 replicate wells for each run. Each run was repeated twice for a total of 20 replicates per sample. This was repeated on a second lot of the NTx Serum. The results of this lot-to-lot precision study are depicted in the table below:

	ZEUS ELISA NTx Serum Test System Lot 1			ZEUS ELISA NTx Serum Test System Lot 2			Both Lots Combined		
	Average (nM BCE)	Standard Deviation	Percent CV	Average (nM BCE)	Standard Deviation	Percent CV	Average (nM BCE)	Standard Deviation	Percent CV
Sample 1	31.6	1.74	5.5%	27.8	3.79	13.7%	29.8	3.49	11.7%
Sample 2	20.3	0.90	4.4%	16.0	1.54	9.6%	18.2	2.47	13.6%
Sample 3	15.2	1.48	9.7%	11.3	1.12	9.9%	13.2	2.31	17.5%
Sample 4	8.6	0.80	9.3%	6.4	0.81	12.6%	7.5	1.37	18.4%

Dilutional Linearity Study:

A sample that was near the upper end of the reportable range of the assay was selected for this study. This sample was diluted to various concentrations using the assay calibrator diluent as a 0 nM BCE diluent. The results of this dilutional linearity study are depicted below:

Amount of Serum Sample	Amount of Calibrator Diluent	Result (nM BCE)	Expected or Theoretical Result
100%	0%	33.6	33.6
90%	10%	30.3	30.2
80%	20%	23.4	26.9
70%	30%	20.8	23.5
60%	40%	17.1	20.2
50%	50%	14.3	16.8
40%	60%	10.1	13.4
30%	70%	7.7	10.1
20%	80%	5.4	6.7
10%	90%	1.2	3.4
0%	100%	0.0	0.0



Potential Biotin Interference:

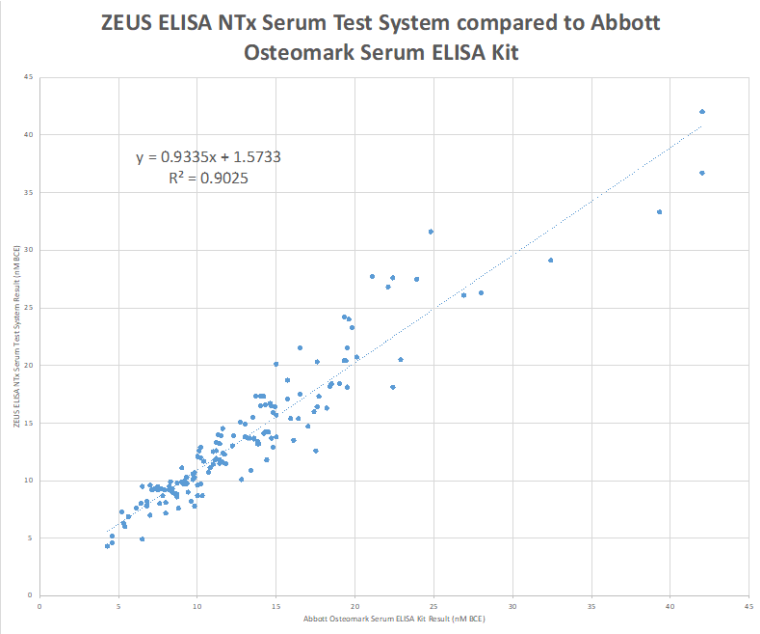
Circulating serum concentrations of biotin in the general population range from 0.1 ng/mL to 0.8 ng/mL (4). When taking extremely high doses of biotin, levels as high as 184 ng/mL have been rarely observed (4). To assess the potential interference of dietary biotin on this assay, a controlled study was performed. Four human serum samples ranging in NTx activity from ~7 nM BCE to ~30 nM BCE were spiked with biotin at the following concentrations: 0.05 ng/mL, 0.10 ng/mL, 0.50 ng/mL, 1.00 ng/mL, 10.0 ng/mL, 20.0 ng/mL, 50 ng/mL, 100 ng/mL and 200 ng/mL. The spiked specimens were tested in duplicate along with their respective un-spiked control sera. This test was repeated on four separate occasions. The percentage difference, spiked versus control was calculated. There were no observable trends or interference observed. It is therefore concluded that dietary biotin supplements should not interfere with the performance of this ELISA test system.

Comparative Study:

The NTx Serum was compared to the Abbott Osteomark® Test Kit in a comparative study. For this investigation, 150 serum specimens were prospectively collected from the Northeastern US. These specimens were collected as follows:

Category	n =	Average Age	Min Age	Max Age	Median Age
Males, < 25 YOA	25	20.36	18	25	19
Males, 26-50 YOA	25	40.2	27	49	42
Males, > 50 YOA	25	65.88	52	78	67
Females, 18-35 YOA	25	24.08	18	34	22
Females, >50 YOA	50	62.74	51	87	62.5

The specimen cohort was tested on both the NTx Serum and Abbott NTx ELISA products in duplicate. The mean of the duplicate values was calculated and plotted in a regression plot. The results of that comparative analysis are depicted below:



This study demonstrates that the NTx Serum is comparable to the Abbott Osteomark® NTx Serum kit across the entire reportable range of the assay.

REFERENCES

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2. Greenblatt MB, Tsai JN, Wein MN. Bone Turnover Markers in the Diagnosis and Monitoring of Metabolic Bone Disease. Clin Chem. 2017 Feb;63(2):464-474. doi: 10.1373/clinchem.2016.259085. Epub 2016 Dec 9. PMID: 27940448; PMCID: PMC5549920.
3. Jain S, Camacho P. Use of bone turnover markers in the management of osteoporosis. Curr Opin Endocrinol Diabetes Obes. 2018 Dec;25(6):366-372. doi: 10.1097/MED.0000000000000446. PMID: 30299435.
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GLOSSARY OF SYMBOLS

The following symbols **may** have been used in the labelling of this product or products associated with this product.

Symbol	Description	Symbol	Description
	Manufacturer		Keep away from sunlight
IVD	*In vitro* diagnostic medical device	PLATE	Plate
REF	Catalogue number	CONJ	Conjugate
	Sufficient for *n* tests	SOLN STOP	Stopping Reagent
LOT	Batch code	WASHBUF 30X	Wash Buffer Concentrate (30X)
	Use by	CONTROL I	Level I Control
	Temperature limitation	CONTROL II	Level II Control
CONT	Contents	CHROMOGEN	Chromogen Reagent
UDI	Unique Device Identifier	CONJ DIL	Conjugate Diluent
	Consult the warnings and precautions	DILSPE	Specimen Diluent
	Consult electronic instructions for use	BUFFER	Buffered Substrate
	Store in the upright position	CAL 0nM	0 nM BCE Calibrator
RX Only	Applicable for U.S.A: Prescription *in vitro* diagnostic product	CAL 5nM	5 nM BCE Calibrator
	Corrosive	CAL 10nM	10 nM BCE Calibrator
	Hazardous Communication	CAL 20nM	20 nM BCE Calibrator
EN	English	CAL 40nM	40 nM BCE Calibrator
		Made in the USA	Made in the USA

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