

# Human N-terminal Telopeptide of Collagen Type-I (NTX) ELISA

to determine Human NTX in Serum, Blood Plasma, Saliva, Urine, Tissue  
Liquid Samples or related Biological Solutions.

## INSTRUCTION MANUAL

FOR ELISA KIT No: LT83826ETKKBA



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## INTENDED USE

This kit is used to determine Human NTX in the sample of serum, blood plasma, saliva, urine, tissue liquid samples or related biological solutions. For *in vitro* use only.

MANUAL VERSION 1.02

## **ASSAY SUMMARY**

A number of sensitive and specific biomarkers have been studied with regard to monitoring bone loss, predicting fracture risk, and differential diagnosis of metabolic bone diseases. The main biomarkers are BSAP, CTX, NTX, and TRACP 5b. Among them, NTX is a stable peptide excreted in the urine after bone resorption. Theoretically, a high serum NTX concentration is highly specific for bone resorption. It has been found that the serum NTX concentration was a sensitive indicator of bone resorption and NTX concentration was negatively correlated with BMD and that a high baseline serum NTX concentration indicated a more rapid decline in bone density. Human N-terminal Telopeptide of Collagen Type-I (NTX) ELISA Kit employs a two-site sandwich ELISA to quantitate NTX in samples. An antibody specific for NTX has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any NTX present is bound by the immobilized antibody. After removing any unbound substances, HRP-Conjugated Human NTX detection antibody is added to the wells. Following a wash to remove any unbound HRP reagent, a Chromogen solution is added to the wells and color develops in proportion to the amount of NTX bound in the initial step. The color development is stopped and the intensity of the color is measured.

## **CHARACTERISTICS**

- This Kit allows for the determination of NTX concentrations in Human serum, cell culture supernates and other biological fluids.
- Detection range: 1.5 nmol/L - 24 nmol/L.
- The minimum detectable dose (MDD) of Human NTX is typically less than 0.1 nmol/L.
- Four samples of known concentration were tested twenty times on one plate to

assess intra-assay precision. The CV (%) < 9%.

□ Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components. The CV (%) < 11%.

□ To assess linearity of the assay, samples containing and/or spiked with high concentrations of Human NTX were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

□ Human N-terminal Telopeptide of Collagen Type-I (NTX) ELISA Kit has high sensitivity and excellent specificity for detection of Human NTX. No significant cross-reactivity or interference between Human NTX and analogues was observed.

## PRODUCT INFORMATION

### MATERIALS SUPPLIED AND STORAGE CONDITIONS

Store kit reagents at 2-8 °C. Immediately after use remaining reagents should be returned to cold storage at 4 °C.

components	48T	96T	Storage conditions
Human NTX microplate	48 wells	96 wells	2-8 °C <sup>1</sup>
Human NTX standard	0.5 mL	0.5 mL	2-8 °C
HRP-Conjugated Human NTX detection antibody	3 mL	6 mL	2-8 °C
Standard diluent	1.5 mL	1.5 mL	2-8 °C
Sample diluent	3 mL	6 mL	2-8 °C
Chromogen solution A	3 mL	6 mL	2-8 °C
Chromogen solution B	3 mL	6 mL	2-8 °C
Stop solution	3 mL	6 mL	2-8 °C
Wash buffer	20 mL (20×)	20 mL (30×)	2-8 °C
Plate covers	1	2	RT
Booklet	1	1	RT

<sup>1</sup>Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal.

May be stored for up to 1 month at 2-8 °C.

## **MATERIALS REQUIRED BUT NOT SUPPLIED**

1. 37 °C incubator
2. Microplate reader
3. Precision pipettes and tips
4. Distilled water
5. Disposable tubes for sample dilution
6. Absorbent paper

## **ASSAY PROTOCOL**

### **SAMPLE COLLECTION AND STORAGE**

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

**Tissue homogenates** - For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS with a glass homogenizer on ice. (The volume depends on the weight of the tissue, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5 minutes at 5000×g to collect the supernate.

**Cell culture supernatants and other biological fluids** - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

**Serum** - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4 °C before centrifugation for 20 minutes at

approximately 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

**Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8 °C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

**Note:** Samples should be centrifuged adequately and no hemolysis or granule was allowed.

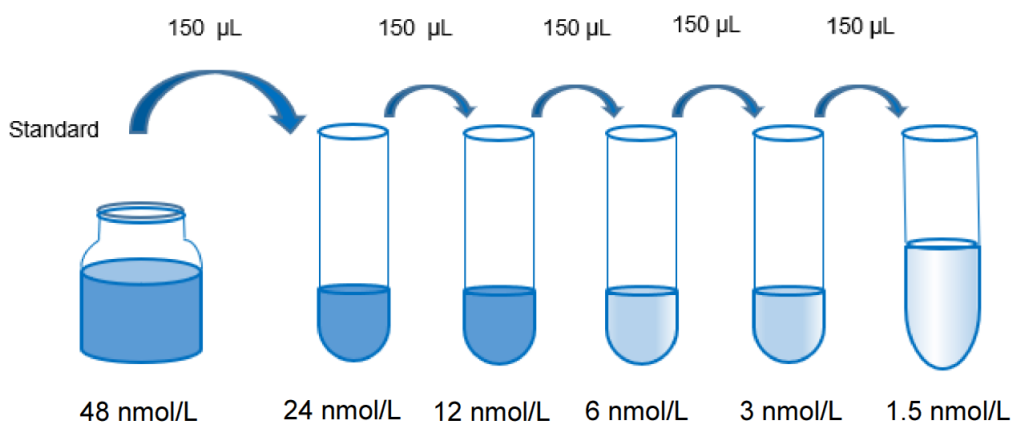
## REAGENT PREPARATION

Bring all reagents to room temperature before use. If crystals were formed in the Buffer Concentrates, warm them gently until they completely dissolved.

**Wash buffer** - Dilute with Distilled or deionized water 1:20 (48T) /1:30 (96T).

**Standard** - Pipette 150 µL of Standard Diluent into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard.

Note: If samples generate values higher than the highest standard, please dilute the samples with Sample Diluent and repeat the assay.



## ASSAY PROCEDURE

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the microplate.
2. Add standard: Set Standard wells, testing sample wells. Add diluted standard 50  $\mu\text{L}$  to standard well.
3. Add Sample: Add sample diluent 40  $\mu\text{L}$  to testing sample well. Then add sample 10  $\mu\text{L}$  to testing sample well, Blank well doesn't add anything.
4. Cover with a plate cover and incubate for 45 minutes at 37 °C.
5. Aspirate each well and wash, repeating the process four times for a total of five washes, 1-3 minutes per time. Wash by filling each well with Wash buffer (250  $\mu\text{L}$ ) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add HRP-Conjugated detection antibody 50  $\mu\text{L}$  to each well, except blank well.
7. Cover with plate cover. Incubate for 30 minutes at 37 °C.
8. Repeat the aspiration/wash process for five times as in step 5.
9. Add chromogen solution A 50  $\mu\text{L}$  and chromogen solution B 50  $\mu\text{L}$  to each well. Gently mix and incubate for 15 minutes at 37°C. Protect from light.
10. Add 50  $\mu\text{L}$  Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

## CALCULATION

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the concentration on the x-axis against the mean absorbance for each standard on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Human NTX concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## **PRECAUTIONS**

1. Do not mix or substitute reagents with those from other lots or sources.
2. This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.
3. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
4. When mixing or reconstituting protein solutions, always avoid foaming.
5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
6. When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees

between wash steps may improve assay precision.

7. Stop Solution should be added to the plate in the same order as the Chromogen solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Chromogen solution.
8. Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.

**VALIDITY & STORAGE:** 12 months (at 2-8°C, unopened).