Type I Collagen N-Terminal Telopeptide (NTX-I) Detection ELISA Kit

Catalog # 6040

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kit to quantify NTX-I fragments/peptides
FORMAT:	Pre-coated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	4 hours
STANDARD RANGE:	20 ng/ml to 0.31 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Urine, Serum, and Plasma
RECOMMENDED SAMPLE DILUTIONS:	1:1 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C
VALIDATION DATA:	Intra-Assay (0.8-7.4%)/Inter-Assay (4.8-6.9%)/Spiking Test (92-102%)
NOTES:	

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INTRODUCTION

Collagen is the most abundant protein in the mammalian body and lends structural integrity to tissues as the primary component of the extracellular matrix (1). Among the many types of collagen, Type I collagen is the main component of bone, tendon, skin, and other tissues (2). In fact, type I collagen makes up 20% of bones by mass, accounting for more than 90% of the organic components. As a result, degraded type I collagen peptides appear in serum and urine in early stages of bone loss or metabolism, and indicate bone turnover levels (3, 4). Proteinases mediate resorption of type I collagen from bone and generate specific peptides of degraded collagen. For example, matrix metalloproteinases (MMPs) exclusively produce C-terminal degraded peptides, ICTP, from type I collagen, while cathepsin K produces CTX-I peptides from the C-terminus and NTX-I peptides from the N-terminus of type I collagen (3).

Patients with osteoporosis display reduced bone mass, indicating that bone cell destruction is greater than bone cell production. In fact, bone loss is increased by lower levels of estrogen after menopause. During the progression of osteoporosis, ICTP and NTX-I are observed in serum as well as urine, and are useful as markers of osteoporosis (4-6). Additionally, bone metastasized cancers, such as breast cancer, prostate carcinoma, and lung cancer, show increased NTX-I levels in serum and urine due to high levels of degraded type I collagen (7, 8).

Thus, degraded peptides of type I collagen are useful tools for evaluating bone metabolism levels in many diseases not only in human patients, but also in mouse disease models of cancer, osteoporosis, osteoarthritis, and rheumatoid arthritis. Chondrex, Inc. provides a mouse NTX-I Detection ELISA Kit (Cat # 6040) as well as a CTX-I Detection ELISA Kit (Cat # 6033) and a Creatinine Assay Kit (Cat # 6041) which is useful for normalizing results among urine samples. Please contact support@chondrex.com or visit www.chondrex.com for more information.

KIT COMPONENTS

Item	Quantity	Amount	Storage
NTX-I Standard (60401)	1 vial	20 ng, lyophilized	-20°C
Detection Antibody (60403)	1 vial	100 µl	-20°C
Solution B - Sample/Standard/Detection Antibody Dilution Buffer (67015)	1 bottle	50 ml	-20°C
Solution D – Streptavidin Peroxidase Dilution Buffer (9055)	1 bottle	20 ml	-20°C
Streptavidin Peroxidase (9029)	2 vials	50 µl	-20°C
TMB (90023)	2 vials	0.2 ml	-20°C
Chromogen Dilution Buffer (90022)	1 bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid (9016)	1 bottle	10 ml	-20°C
Wash Buffer, 20X (9005)	1 bottle	50 ml	-20°C
Capture Antibody Coated 96-Well ELISA Plate (Pink)	1 each	8-well Strips x12	-20°C

ASSAY OUTLINE

Add 100 µl of diluted standards and samples into wells

↓ Incubate at room temperature for 2 hours.
Wash plate.

Add 100 µl of diluted Detection Antibody

↓ Incubate at room temperature for 1 hour.
Wash plate.

Add 100 µl of diluted Streptavidin Peroxidase

↓ Incubate at room temperature for 30 minutes.
Wash plate.

Add 100 µl of TMB solution

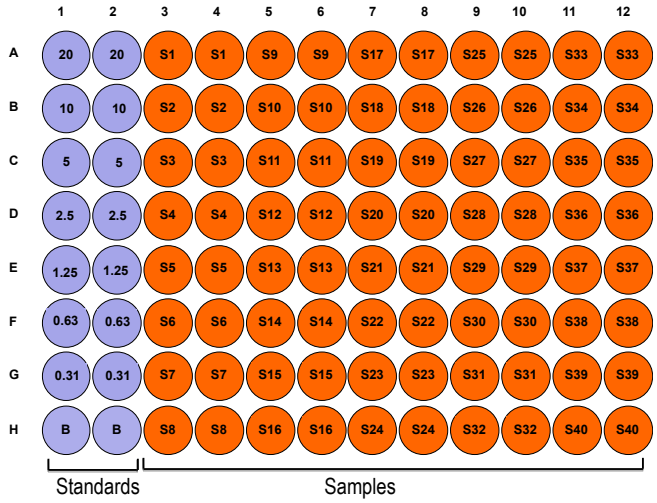
↓ Incubate at room temperature for 25 minutes.

Add 50 µl of Stop Solution

↓

Read plates at 450 nm/630 nm

PLATE MAPPING



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NOTES BEFORE USING ASSAY

NOTE 1: It is recommended that the standard and samples be run in duplicate.

NOTE 2: Warm up all buffers to room temperature before use.

NOTE 3: Crystals may form in Wash Buffer, 20X when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are completely dissolved.

NOTE 4: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

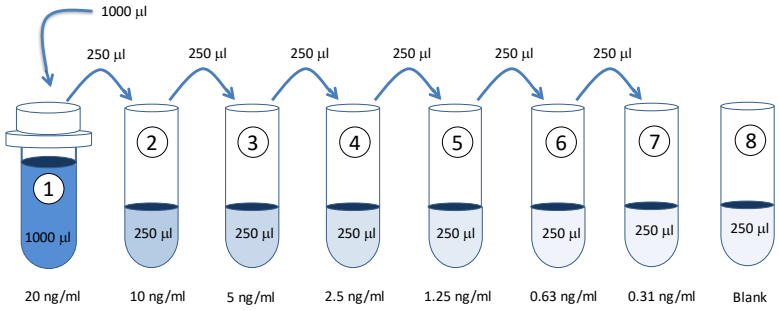
NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

NOTE 6: For partial reagent use, please see the assay protocol's corresponding step for the appropriate dilution ratio. For example, if the protocol dilutes 50 µl of a stock solution in 10 ml of buffer for 12 strips, then for 6 strips, dilute 25 µl of the stock solution in 5 ml of buffer. Partially used stock reagents may be kept in their original vials and stored at -20ºC for use in a future assay.

NOTE 7: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

ASSAY PROCEDURE

1. **Prepare Standard Dilutions**: The recommended standard range is 0.31 - 20 ng/ml. Dissolve one vial of NTX-I standard in 1 ml of Sample/Standard/Detection Antibody Dilution Buffer (Solution B) for the 20 ng/ml standard. Then serially dilute it with Solution B. For example, mix 250 µl of the standard (20 ng/ml) with an equal volume of Solution B to make a 10 ng/ml solution, and then repeat it five more times for 5, 2.5, 1.25, 0.63, and 0.31 ng/ml solutions. The remaining 20 ng/ml standard stock may be stored at -20°C for use in a second assay. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



2. **Prepare Sample Dilutions:** Centrifuge samples at 10,000 rpm at 4°C for 3 minutes to remove insoluble materials and lipids. Dilute the supernatants with an equal volume of Solution B. For example, take 100 µl of supernatant, and mix with 100 µl of Solution B. If the OD values of samples are higher than the OD values of the 20 ng/ml standard, re-assay the sample at a higher dilution.

   NOTE: Dilute samples at least 1:1 with Solution B depending on the estimated NTX-I level in the samples. Two to three different sample dilutions are recommended if the NTX-I levels in the samples are unknown.

3. **Add Standards and Samples**: Add 100 µl of Solution B (blank), standards, and samples to designated wells. Incubate at room temperature for 2 hours.

4. **Dilute Wash Buffer**: Dilute 50 ml of Wash Buffer, 20X in 950 ml of distilled water (1X wash buffer). Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

5. **Add Detection Antibody Solution**: Prepare detection antibody solution with Solution B as shown in the following table. Add 100 µl of detection antibody solution to each well and incubate at room temperature for 1 hour.

Strip #	Detection Antibody (µl)	Solution B (ml)
2	17	1.7
4	25	3.3
6	50	5.0
8	66	6.6
10	82	8.2
12	100	10.0

6. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

7. **Add Streptavidin Peroxidase Solution**: Prepare streptavidin peroxidase solution with Streptavidin Peroxidase Dilution Buffer (Solution D) as shown in the following table. Add 100 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 30 minutes.

Strip #	Streptavidin Peroxidase (µl)	Solution D (ml)
2	8.5	1.7
4	17	3.3
6	25	5.0
8	33	6.6
10	41	8.2
12	50	10.0

8. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

9. **Add TMB Solution**: Use new tubes when preparing TMB solution. Just prior to use, prepare TMB solution with Chromogen Dilution Buffer as shown in the following table. Add 100 µl of TMB solution to each well immediately after washing the plate and incubate for 25 minutes at room temperature.

Strip #	TMB (µl)	Chromogen Dilution Buffer (ml)
2	34	1.7
4	66	3.3
6	100	5.0
8	132	6.6
10	164	8.2
12	200	10.0

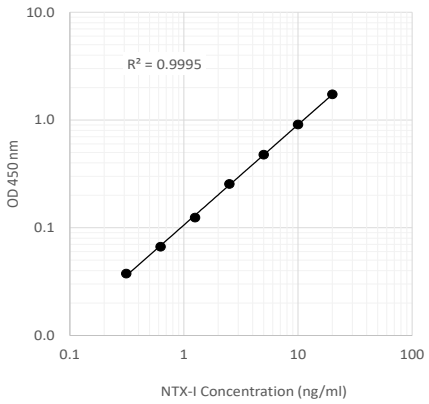
10. **Stop**: Stop the reaction with 50 µl of 2N Sulfuric Acid (Stop Solution) to each well.

11. **Read Plate**: Read the OD values at 450 nm. If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution. A 630 nm filter can be used as a reference.

CALCULATING RESULTS

1. Average the duplicate OD values for the blank, standards, and test samples.
2. Subtract the “blank” (B) values from the averaged OD values in step 1.
3. Plot the OD values of standards against the concentration of NTX-I (ng/ml). Using a log/log plot will linearize the data. Figure 1 shows a representative experiment where the standard range is 0.31 - 20 ng/ml.
4. The ng/ml of NTX-I in test samples can be calculated using regression analysis.

Figure 1 - A Typical Standard Curve for the NTX-I Detection ELISA Kit



VALIDATION DATA

Table 1 – Reproducibility Data for the NTX-I Detection ELISA Kit

Test	0.63 ng/ml	2.5 ng/ml	10 ng/ml
Intra-Assay CV (%)	7.4	1.8	0.8
Inter-Assay CV (%)	4.8	5.6	6.9
Spike Test* (%)	92%	97%	102%

* Known amounts of NTX-I were added to samples and then diluted with Sample/Standard/Detection Antibody Dilution Buffer (Solution B).

TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.’s ELISA FAQ for more information.

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