

Type I Collagen C-telopeptide, CTX-I, Assay Kit

Catalog # 6033

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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). 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, which accounts for more than 90% of the organic components. As a result, degradation products of type I collagen can be detected in serum and urine in stages of bone loss or metabolism and can be potential markers of bone metabolism (3).

Proteinases mediate resorption of type I collagen from bone and generate specific peptide fragments of degraded collagen. For example, matrix metalloproteinases (MMPs) exclusively produce C-terminal degraded fragments (ICTP) from type I collagen, while cathepsin K produces CTX-I fragments from the C-terminus and NTX-I fragments from the N-terminus of type I collagen (4). Because the proteinase activities differ among diseases and degraded fragments reflect the metabolism of type I collagen, immunoassays have been developed to monitor the levels of these degraded fragments in biological fluids (5).

Patients with osteoporosis display reduced bone mass. During disease progression, degraded peptides of type I collagen are observed in serum as well as urine. Therefore, ICTP and NTX-I have been used as markers of osteoporosis (6-8). In addition, it was reported that CTX-I levels in urine are correlated with disease activity of osteoarthritis (9). Furthermore, cancers which metastasize to bone can affect the metabolism of type I collagen. Serum CTX-I levels correlate with prognosis of these cancers, especially prostate, lung, breast, and urinary bladder cancers (10-13).

Thus, degraded type I collagen fragments are very useful tools for evaluating disease not only in humans, but also in mice, leading to the development of many mouse disease models for cancer, osteoporosis, osteoarthritis, and rheumatoid arthritis. Chondrex, Inc. has developed a CTX-I detection ELISA kit for mouse and human using a competitive assay system with a monoclonal antibody which recognizes conserved peptide sequences in mouse and human (13). Chondrex, Inc. is also in the process of developing other type I collagen peptide detection kits such as a NTX-I detection kit. Contact support@chondrex.com for more information.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Capture Antibody (60332)	1 vial	100 µl	-20°C
Standard C-Telopeptide, CTX-I (60331)	1 vial	100 µl	-20°C
Biotinylated C-Telopeptide, CTX-I (60333)	1 vial	100 µl	-20°C
Solution A - Capture Antibody Coating Buffer (9052)	1 bottle	10 ml	-20°C
Solution B - Sample/Standard 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 Solution (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
96-well ELISA Plate	1 each	96-well, (8-well strips x 12)	-20°C
Flexible 96-well Plate	1 each	96-well	-20°C

CTX-I Assay Outline

96-well ELISA plate

Step 1 Add 100 μ l of Capture Antibody solution to the 96-well ELISA plate

Incubate at 4°C overnight.
Wash plates.

Flexible 96-well plate

Step 2

Add 75 μ l of diluted standards and samples

+

Add 75 μ l of diluted Detection Antibody

Mix the solution in a flexible 96-well plate.

Then, transfer 100 μ l of the mixture into the
Capture Antibody coated ELISA plate
according to the plate mapping figure.

Incubate at room temperature for 2 hours.
Wash plates.

Step 3 Add 100 μ l of Streptavidin Peroxidase

Incubate at room temperature for 30 minutes.
Wash plates.

Step 4 Add 100 μ l of TMB solution

Incubate plate at room temperature for 25 minutes.

Step 5 Add 50 μ l of Stop Solution

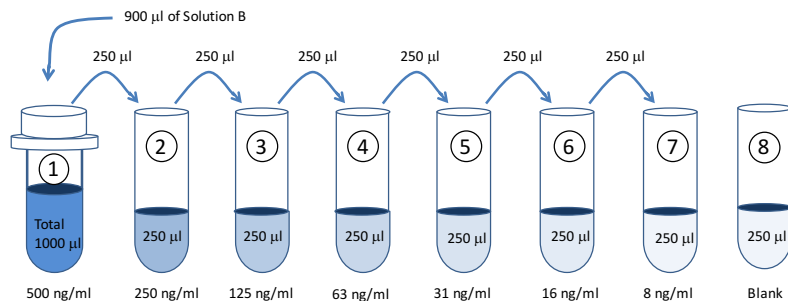
Step 6 Read plates at 450 nm/630 nm

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: Partially used reagents may be kept at -20°C .
- Note 4: Crystals may form in the 20X wash buffer 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 5: Measure exact volume of buffers using a serological pipette as extra buffer is provided.
- Note 6: Cover the plate with plastic wrap or a plate sealer after each step to avoid the edge effect.

ASSAY PROCEDURE

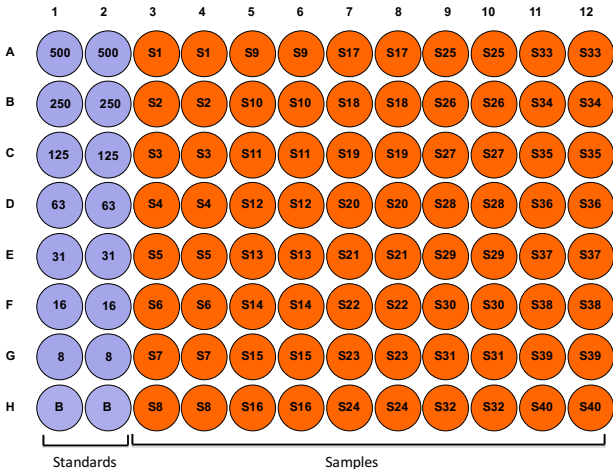
- Add Capture Antibody:** Dilute one vial of Capture Antibody with 10 ml of Capture Antibody Dilution Buffer (Solution A). Add 100 μl of capture antibody solution to each well and incubate at 4°C overnight.
- Dilute Wash Buffer:** Dilute 50 ml of 20X wash buffer in 950 ml of distilled water (1X wash 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 blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*
- Prepare Standard Dilutions:** The recommended CTX-I standard range is 8 - 500 ng/ml. Add 900 μl of Sample/Standard Dilution Buffer (Solution B) to one vial of Standard (500 ng/vial) and keep it as a 500 ng/ml standard stock. Then, serially dilute it with Solution B. For example, mix 250 μl of the 500 ng/ml solution with an equal volume of Solution B to make a 250 ng/ml solution, and then repeat it five more times for 125, 63, 32, 16, and 8 ng/ml standard solutions.



- Prepare Sample Dilutions:** Sample dilution (serum or urine) varies (1:100 or more) depending on the disease and timing of serum collection. In general, no CTX-I is observed in normal serum at a 1:100 dilution.
- Prepare biotinylated CTX-I :** Dilute one vial of biotinylated c-telopeptide with 9.9 ml of Sample/Standard Dilution Buffer (Solution B).

- Add Standards and Samples:** Mix 75 μl of standards, diluted sample, and Solution B (blank) with 75 μl of diluted biotinylated C-telopeptide in the flexible plate provided. Then transfer 100 μl of the mixture to the wells of the capture antibody coated plate from Step 1. Incubate at room temperature for 2 hours.

Figure 1 - CTX-I Assay Standard layout.



- 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 blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*
- Add Streptavidin Peroxidase:** Dilute one vial of Streptavidin Peroxidase in 10 ml of Streptavidin Peroxidase Dilution Buffer (Solution D). Add 100 μl of streptavidin peroxidase solution to each well and incubate at room temperature for 30 minutes.
- 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 blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*
- Add TMB:** Use new tubes when preparing TMB. Dilute one vial of TMB with 10 ml Chromogen Dilution Buffer just prior to use. Add 100 μl of TMB solution to all wells immediately after washing the plate and incubate for 25 minutes at room temperature.
- Stop:** Add 50 μl of 2N sulfuric acid (Stop Solution) to each well.
- Read Plate:** Read the OD values at 450 nm (a 630 nm filter can be used as a reference).

NOTE: This is a competitive assay. Sample re-assay is necessary if the samples show the following results.

- If the OD values of samples are lower than the OD values of the highest standard, re-assay the samples at a higher dilution.
- If the OD values of samples are higher than the OD values of the lowest standard, re-assay the samples at a lower dilution.

CALCULATION OF ANTIBODY TITERS

1. Average the duplicate OD values for the standards, blanks (B), and test samples.
2. Calculate the ratio of OD values of each standard or sample against the OD values of the blank (B).

Ratio = OD values of standard/samples / OD value of blank.

3. Plot the ratio of the standards against the ng/ml of C-telopeptide standard. Using a log/log plot will linearize the data. Figure 2 shows an example of a standard curve of CTX-I.

NOTE: A calculation spread sheet can be downloaded from Chondrex, Inc.'s website.

4. The ng/ml of antibodies in test samples can be calculated using regression analysis. Multiply the ratio of the sample OD values by the sample dilution factor to obtain the CTX-I concentration (ng/ml) in original sample specimens.

Figure 2 - A Typical Standard Curve for a CTX-I Assay

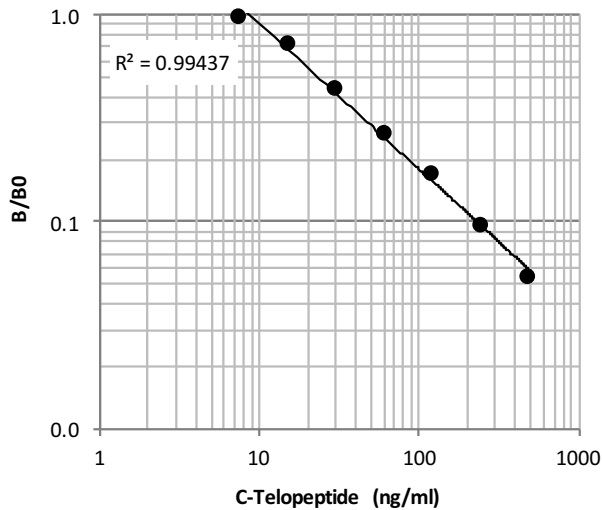


Table 1 - Reproducibility of data assayed by CTX-I Assay Kit

Test At	16 ng/ml	63 ng/ml	250 ng/ml
Inter-Assay CV (%)	7.2	5.5	10.0
Intra-Assay CV (%)	1.8	2.2	1.6
Spiking Test*	108%	113%	109%

Known amounts of CTX-I were added to samples and then diluted with Sample/Standard Dilution Buffer to assay CTX-I by ELISA.

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