Type I Collagen C-Terminal Telopeptide (CTX-I) Detection ELISA Kit

Catalog # 6033

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA Kit to quantify CTX-I fragments/peptides
FORMAT:	96-well ELISA Plate with removeable strips
ASSAY TYPE:	Competitive ELISA
ASSAY TIME:	3 hours
STANDARD RANGE:	500 - 8 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Urine, Serum, and Plasma
RECOMMENDED SAMPLE DILUTIONS:	1:100 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	Intra-Assay (1.6-2.2%)/Inter-Assay (5.5-10%)/Spiking Test (108-113%)
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). 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 correlate 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 (Cat # 6033) for mouse and human samples using a competitive assay system with a monoclonal antibody which recognizes conserved peptide sequences in mouse and human (13).

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 - 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

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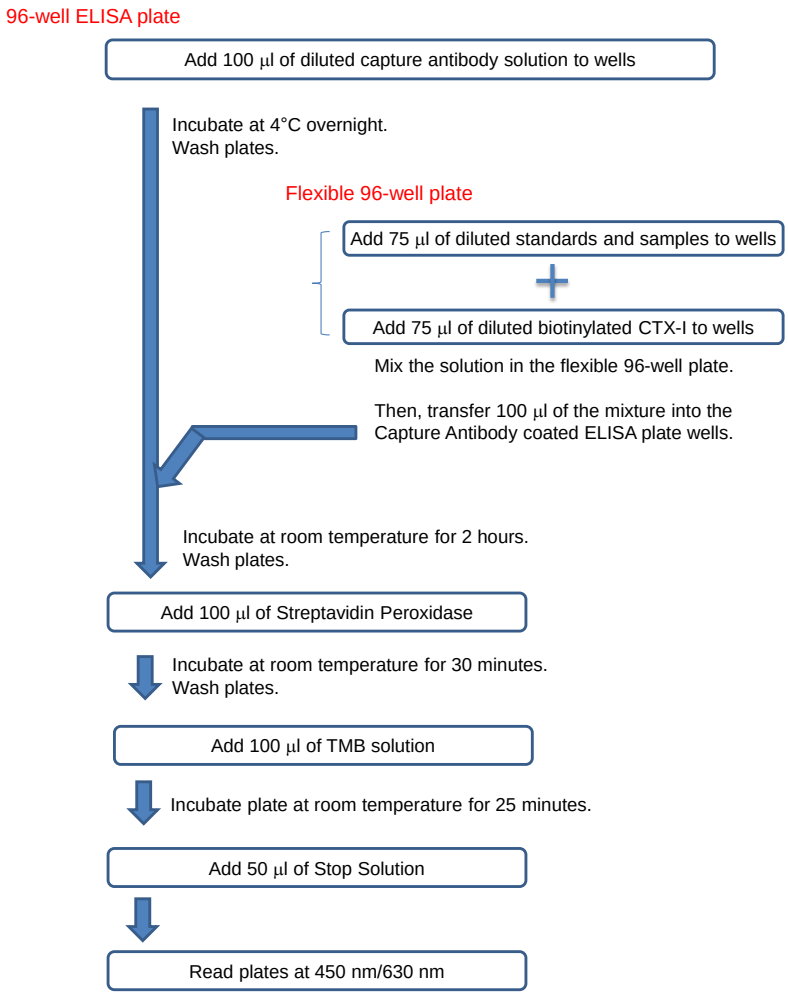
ASSAY OUTLINE



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 -20°C 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.

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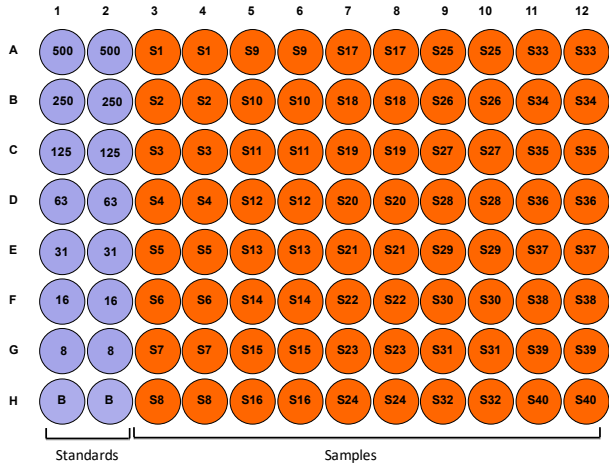
PLATE MAPPING

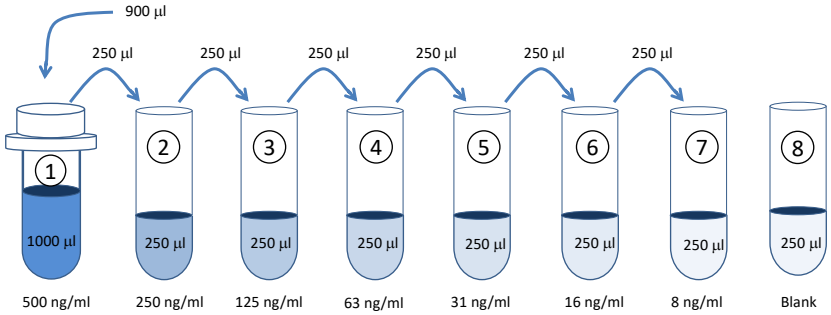


ASSAY PROCEDURE

1. **Add Capture Antibody**: Dilute one vial of Capture Antibody with 10 ml of Coating Buffer (Solution A). Alternatively, dilute according to the table below. Add 100 µl of capture antibody solution to each well and incubate at 4°C overnight. Any leftover Capture Antibody Stock Solution may be stored at -20°C for future assays.

Strip #	Capture Antibody (µl)	Solution A (ml)
2	17	1.7
4	33	3.3
6	50	5.0
8	66	6.6
10	82	8.2
12	100	10.0

2. **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 solution.



3. **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.

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4. **Prepare Biotinylated CTX-I**: Dilute one vial of biotinylated c-telopeptide with 10 ml of Sample/Standard Dilution Buffer (Solution B).

Strip #	Biotinylated CTX-I (µl)	Solution B (ml)
2	17	1.7
4	33	3.3
6	50	5.0
8	66	6.6
10	82	8.2
12	100	10.0

5. **Mix Standards, Samples, and Biotinylated CTX-I**: Using the Plate Mapping figure as a guide, mix 75 µl of standards, diluted sample, and Solution B (blank) with 75 µl of diluted biotinylated C-telopeptide in the flexible plate provided.

6. **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 blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

7. **Transfer Mixtures**: Transfer 100 µl from the wells of the flexible plate to the corresponding wells of the washed capture antibody coated plate. Incubate at room temperature for 2 hours.

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 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.

Strip #	Streptavidin Peroxidase (µl)	Solution D (ml)
2	8	1.7
4	17	3.3
6	25	5.0
8	33	6.6
10	42	8.2
12	50	10.0

10. **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.*

11. **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.

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

12. **Stop**: Add 50 µl of 2N sulfuric acid (Stop Solution) to each well.

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13. **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. Re-assaying samples may be necessary if the samples show the following results.

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

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

CALCULATING RESULTS

1. Average the duplicate OD values for the blank, standards, and test samples.

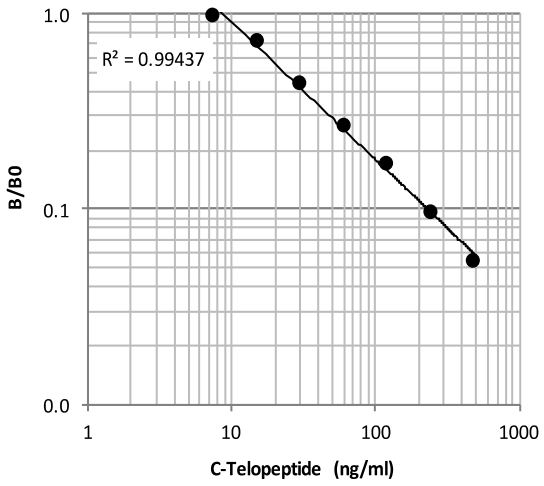
2. Calculate the ratio of OD values of each standard or sample against the OD values of the blank (B).

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

3. Plot the OD values of standards against the concentration of C-telopeptide standard (ng/ml). Using a log/log plot will linearize the data. Figure 1 shows a representative experiment where the standard range is 8 - 500 ng/ml.

4. The ng/ml of C-telopeptides 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. For additional assistance, please download a sample calculation worksheet from www.chondrex.com.

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



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ASSAY VALIDATION

Table 1 - Reproducibility Data for the CTX-I Detection ELISA Kit

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

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

TROUBLESHOOTING

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

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