Horse Cross-Linked C-Terminal Telopeptides of Type II Collagen (CTX-II) ELISA Kit

Cat.No: AAA10242

Store the kit at 2°C-8°C !

Valid Period: Six Months (2°C-8°C)!

Specification: 48 well kit or 96 well kit

FOR LAB REAGENT/RESEARCH USE ONLY!

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!

1. Introductions

This **Quantitative Sandwich ELISA** kit is for lab reagent/research use only, not for drug, household, therapeutic or diagnostic applications! This kit is intended to be used for determine the level of CTX-II (hereafter termed "analyte") in **undiluted original Horse serum, plasma or tissue homogenates samples**. For other sample types please contact tech support to determine compatibility with this assay. This kit is not suitable for assaying non-biological sources of substances.

2. Performances

Sensitivity: The sensitivity of this kit is 10pg/ml.

Detection Range: The detection range of this kit is 125pg/ml-4000pg/ml.

Standard Concentration Gradients (S6 to S1) : 4000,2000,1000,500,250,125pg/ml.

Reproducibility: Both Intra-assay CV (%) and Inter-assay CV (%) is less than 15%. [CV(%) = SD/mean ×100]. All CV% should be compared by concentration, not compared by OD values.

3. Precautions

3.1) We reserve the right to amend the terms and conditions of any change without prior notice in this manual.

3.2) Limited by current skill and knowledge, it is impossible for us to complete the cross-reactivity detection between this analyte and all its analogues; therefore, cross reaction to other targets may potentially exist. Furthermore, cross-reactivity could vary between sample type or species.

3.3) Influenced by factors including cell viability, cell number and sampling time, samples from cell culture supernatant/extract or cell lysates are not suitable for detection by this kit.

3.4) Pure buffer controls are not suitable for this one step assay because they do not reflect the contribution of the biological matrix of an endogenous sample to reducing the background of the assay. If a pure buffer blank is required then please do not add the HRP-Conjugate Reagent to the pure buffer blank reaction mixture.

3.5) The reagents and the plate of the kit and its technical parameters are matched and designed for optimal performance only when they are together as a whole kit, so please do not substitute reagents from one kit to other kits and use only the reagents supplied by manufacturer. Due to the possibility of mismatching between antigen/antibody from other manufacturers and antibody/antigen used in this kit (such as differences in conformational epitopes caused by chemical environments or differences in linear epitopes, and so on), some synthetic peptides, recombinant proteins and extracted proteins can not be detected out by this kit. Additionally, we will not be responsible for using this kit or any part of this kit to do any other experiments (such as western blot, immunohistochemistry, spike/recovery and so on) arbitrarily.

3.6) Results from end users might be inconsistent with our in house data due to some unexpected transportation or storage conditions, or different ambient temperature, lab equipment, operation, pipetting, washing, incubation temperature or time, and kit age. Assay variance among wells or kits might arise from these factors, too.

3.7) The Stop Solution is an acid solution, please avoid contact it, it may cause skin irritations and burns. so please pay enough attention to safety when use it. Samples 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 body will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.

4. Materials Required but Not Supplied

4.1) Distilled water.

4.2) Absorbent paper or paper towels.

4.3) Pipettes and disposable pipette tips.

4.4) An ELISA reader capable of measuring absorbance at 450 nm.

4.5) A constant temperature incubator which can provide stable incubation conditions up to 37°C±0.5°C.

5. Materials Supplied

Items	Materials	Color of covers	48 well kit	96 well kit
1	Microelisa Stripplate	---	48 well plate	96 well plate
2	Standards	S1(Red); S2(Pink); S3(Blue); S4(Green); S5(Yellow); S6(White)	0.5ml×6 vials	0.5ml×6 vials
3	Sample Diluent	Blue	3.0ml×1 bottle	6.0ml×1 bottle
4	HRP-Conjugate Reagent	Red	5.0ml×1 bottle	10.0ml×1 bottle
5	20×Wash Solution	White	15ml×1 bottle	25ml×1 bottle
6	Stop Solution	Yellow	3.0ml×1 bottle	6.0ml×1 bottle
7	Chromogen Solution A	Purple	3.0ml×1 bottle	6.0ml×1 bottle
8	Chromogen Solution B	Black or Brown	3.0ml×1 bottle	6.0ml×1 bottle
9	Closure Plate Membrane	---	2×pieces	2×pieces
10	Manual	---	1×paper	1×paper

6. Samples Collection and Storage

Although we have listed most of possible sample types, it does not mean the analyte exists in all of these listed samples, because some analytes only exist in specific body fluids, organelles, cells or tissues. End users have the responsibility to judge whether their samples contain the analyte.

Serum:	Collect and centrifuge serum at 1000×g (or 3000rpm) for approximately 20 minutes. Collect the supernatant carefully. Assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.
Plasma:	Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge the plasma at 1000×g (or 3000rpm) for approximately 20 minutes. Collect the supernatant carefully. Assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.
Whole Blood:	Collect whole blood using EDTA or heparin as an anticoagulant, and using ultrasonication or two freeze-thaw cycles to break the cell membranes. Centrifuge the whole blood at 1000×g (or 3000rpm) for approximately 20 minutes. Collect the supernatant carefully. Assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.
Other Fluid:	Collect and centrifuge fluid at 1000×g (or 3000rpm) for approximately 20 minutes. Collect the supernatant carefully. Assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.
Tissue:	The preparation of tissue homogenates will vary depending upon tissue type. Collect and weigh tissue before homogenization, mince the tissue to small pieces and homogenize the tissue with a certain amount of PBS (usually 10mg tissue to 100μl PBS.). Centrifuge the homogenate at 1000×g (or 3000rpm) for approximately 20 minutes. Collect the supernatant carefully. Assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.
Saliva:	Collect and centrifuge saliva at 1000×g (or 3000rpm) for approximately 20 minutes. Collect the supernatant carefully. Assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.
Urine:	Collect and centrifuge urine at 1000×g (or 3000rpm) for approximately 20 minutes. Collect the supernatant carefully. Assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.
Feces:	Collect and fully shake feces with a certain amount of PBS (usually 10mg feces to 100μl PBS.). Centrifuge the homogenate at 1000×g (or 3000rpm) for approximately 20 minutes. Collect the supernatant carefully. Assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Important Notes:

6.1) We are only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amounts of samples needed for the entire assay. Please make sure that sufficient samples are available.

6.2) Fresh samples without long time storage are recommended for the assay. Otherwise, protein degradation and denaturation may occur in the samples and lead to wrong results. Samples to be used within a week can be stored at 2°C-8°C, otherwise samples should be stored at -20°C(≤three month) or -80°C(≤six months) to help avoid loss of bioactivity and contamination. Avoid repeated freeze/thaw cycles (less than 3 cycles, and no more than 5 cycles). The validity period of samples may vary depending on the analyte, sample type, storage method and other variables.

6.3) There is no unified rules about how long to clot samples after the blood draw. You can clot for 2 hours at room temperature or overnight at 4°C, but it is not necessary to do it.

6.4) Grossly hemolyzed samples are not suitable for use in this assay, so the samples should be centrifuged adequately and no hemolysis or granule is allowed.

6.5) The kit can not assay samples which contain sodium azide (NaN3), because NaN3 will inhibit the activity of horseradish peroxidase (HRP).

6.6) If sample types are not indicated in the manual, a preliminary experiment to determine the validity of this kit is necessary.

7. Reagent Preparation and Storage

Please store the kits (the Plate and all reagents) at 2°C-8°C.

7.1) The valid period of the unopened kit is up to six months at 2°C-8°C. The kit should not be used beyond the expiration date.

7.2) Wash Solution (1×) - Dilute one volume of Wash Solution (20×) with nineteen volumes of deionized or distilled water. Diluted Wash Solution is stable for one month at 2°C-8°C. Undiluted Wash Solution and other reagents are stable for up to six months at 2°C-8°C.

7.3) When the kit is opened, please use up the plate as soon as possible after removing the plate from the foil pouch, because the plate easily become damp after repeatedly removed it from the foil pouch. The Plate is detachable, so please return the unused wells to the foil pouch containing the desiccant pack, and reseal along the entire edge of the zip-seal for preventing moisture. The remaining reagents also need to be stored at 2°C-8°C. The valid period of opened kits may vary depending on various factors and for best results the kit should be used up as soon as possible.

8. Assay Procedures

Steps ↓	Operations
Step 1	Check the Plate and equipment before your experiments and make sure they are no problem. Check the labels and the color of the covers of the vials/bottles and make sure they are matched and no mistake.
Step 2	Allow the **Plate**, all **reagents** and **samples** to come to room temperature (18°C-25°C) **naturally** before starting assay procedures. Do **NOT** use hot water baths to heat the Plate, reagents or samples.
Step 3	Removed the Plate from the foil pouch. The Plate is detachable, return the unused strips to the foil pouch with the desiccant pack, and reseal for preventing damp.
Step 4	Set Blank wells; Set Standard wells; Set Sample wells.
Step 5	Add **nothing** to **all Blank wells**; Add 50 µl **Standard (S_1, S_2, S_3, S_4, S_5, S_6)** to **corresponding Standard wells**; Add 50 µl **Sample** to **every Sample well**.
Step 6	Add **100 µl HRP-Conjugate Reagent** to **every** well **except blank wells**;
Step 7	Cover the Plate with a Closure Plate Membrane and **incubate** for 60 minutes at 37°C.
Step 8	Wash all wells (including all Blank wells) 4 times.
Step 9	Add 50 µl **Chromogen Solution A** to **every well.**
Step 10	Add 50 µl **Chromogen Solution B** to **every well.** (**Protect Chromogen Solution B from light.**)
Step 11	Mix gently and **incubate** the Plate for 15 minutes at 37°C. (**Protect the Plate from light.**)
Step 12	Add 50 µl **Stop Solution** to **every well**.
Step 13	Read the Optical Density (O.D.) at 450 nm using an ELISA reader **within** 15 minutes after adding Stop Solution (around 5 minutes is usually the best time).

Important Notes:

8.1) Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be covered tightly to prevent evaporation and contamination with microorganisms.

8.2) Do not remove the Plate from the foil pouch until needed. There may be some foggy substance in the wells when the Plate is opened the first time. This will not have any effect on the final assay results.

8.3) The foil pouch may leak and no longer vacuum after stored or transported for a long distance. This will not have any effect on the final assay results.

8.4) Samples or Reagents Addition: Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall in as much as is possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the Plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all blanks, standards and samples, although not required, is recommended. To avoid contamination, please use fresh disposable pipette tips for each transfer.

8.5) Incubation: To ensure accurate results, proper adhesion of the Plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, Do not let the strips dry at any time during the assay. Incubation time and temperature must be observed. Do not shake during incubation, because shaking may affect the binding reaction of antigen and antibody particularly if not shaking evenly.

8.6) Washing Plate: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the Plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading.

Normally there are two ways of washing: **Manual Washing** and **Automated Washing**

Manual Washing - Dump the incubation mixtures of the wells into a sink or proper waste container. Using a pipette or squirt bottle, fill each well completely with Wash Solution (1×), leave the Wash Solution in the wells for about one minute and then invert and hit the Plate onto absorbent papers or paper towels until no moisture appears. Repeat this procedure four times. **Note**: Hold the sides of the Plate frame firmly when washing the Plate to assure that all strips remain securely in Plate frame.

Automated Washing - Aspirate all wells, then wash the Plate four times using Wash Buffer (1×). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350μl/well/wash. After the final wash, invert the Plate, and blot the Plate dry by hitting the Plate onto absorbent paper or paper towels until no moisture appears.

8.7) Controlling Reaction Time: Observe the change of color after adding Substrates (e.g. observation once every 10 minutes). Substrates should change from colorless or light blue to gradations of blue. The color developed in the wells will turn from blue to yellow after adding the Stop Solution.

8.8) Chromogen Solution B is 3,3',5,5'-Tetramethylbenzidine (TMB), and it is easily degraded by sunlight and by fluorescent lights, so please **protect it from light**.

9. Calculation of Results

9.1) Average the duplicate readings for each standard and sample to subtract average optical density of the Blank (OD_0).

Concentration:	**Blank**	**S_1**	**S_2**	**S_3**	**S_4**	**S_5**	**S_6**
Mean O.D.(450nm):	OD_0	OD_1	OD_2	OD_3	OD_4	OD_5	OD_6

9.2) Use the professional curve fitting software to make the standard curve (usually the standard curve is linear, quadratic and cubic curve) and calculate the level of the analyte.

9.3) If the highest OD value of the samples are higher than the highest OD value of the standards, please dilute the samples with equal or double volume of Sample Diluent and repeat the assay again.

Note: Any variation in ambient temperature, equipment, operation, pipetting, washing, incubation temperature or time, and kit age can cause variations in results. Every user should obtain their own standard curve.