Porcine (CTX-Ⅱ)

ELISA Kit Instruction

Catalogue No.

201-10-0012

Preface

Please carefully read this instruction before using. This ELISA kit is based on the principle of double-antibody sandwich technique to detect Porcine (CTX-Ⅱ). Be used only for research purposes, not be used for medical diagnosis.

Full Name

Porcine C-telopeptide of type Ⅱ collagen, CTX-ⅡELISA Kit

Intended Use

This kit is used to assay the C-telopeptide of type Ⅱ collagen, CTX-Ⅱ in the sample of Porcine’s serum, blood plasma, and other related tissue Liquid.

Test principle

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Porcine C-telopeptide of type Ⅱ collagen, CTX-Ⅱin samples. Add C-telopeptide of type Ⅱ collagen, CTX-Ⅱ to

monoclonal antibody Enzyme well which is pre-coated with Porcine C-telopeptide of type Ⅱ collagen, CTX-Ⅱmonoclonal antibody, incubation; then, add (CTX-Ⅱ)antibodies labeled with biotin, and combined with Streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add Chromogen Solution A, B, the color of the liquid changes into the blue, And at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the Porcine Substance C-telopeptide of type Ⅱ collagen, CTX-Ⅱof sample were positively correlated.

Materials supplied in the Test Kit

1	Standard (480ng/ml)	0.5ml
2	Standard diluent	3ml
3	Microelisa Strip plate	12well×8strips
4	Str- HRP-Conjugate Reagent	6ml
5	30×wash solution	20ml
6	Biotin-(CTX-Ⅱ) Ab	1ml
7	Chromogen Solution A	6ml
8	Chromogen Solution B	6ml
9	Stop Solution	6ml
10	Instruction	1
11	Closure plate membrane	2
12	Sealed bags	1

Materials required but not supplied

1. 37 ℃ incubator
2. Standard Enzyme reader
3. Precision pipettes and Disposable pipette tips
4. Distilled water
5. Disposable tubes for sample dilution
6. Absorbent paper

Important Notes

1. Been taken out from the 2-8℃ environment, the kit should be balanced 30 minutes in the ambient temperature then use. If the Coated plates of Enzyme haven't been used up after opened, the remaining plates should be stored in Sealed bag.
2. For each step, add Sample with sample injector which should be calibrated frequently, in order to avoid unnecessary experimental tolerance.
3. he operation shall be carried out accordance to the instructions strictly. And test results must be based on the readings of the Enzyme reader.
4. In order to avoid cross-contamination, it is forbidden to re-use the suction head and seal plate membrane in your hands.
5. All samples, washing buffer and each kind of reject should according to infective material process.
6. The idle agents shall be put up or covered. Do not use reagent with different batches. And use them before expired date.
7. The substrate B is light-sensitive. Prolonged exposure to light is forbidden.

Washing method

Manually washing method: shake away the remain liquid in the enzyme plates; place some bibulous papers on the test-bed, and flap the plates on the upside down strongly. Inject at least 0.35ml after-dilution washing solution into the well, and marinate 1~2 minutes. Repeat this process according to your requirements.

Automatic washing method: if there is automatic washing machine, it should only be used in the test when you are quite familiar with its function and performance.

Specimen requirements

1. Can't detect the sample which contain NaN3, because NaN3 inhibits HRP active
2. extract as soon as possible after Specimen collection, and according to the relevant literature, and should be experiment as soon as possible after the extraction. If it can't, specimen can be kept in -20 ℃ to preserve, Avoid repeated freeze-thaw cycles.
3. serum- coagulation at room temperature 10-20 mins, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.
4. plasma-use suited EDTA or citrate plasma as an anticoagulant, mix 10-20 mins, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.
5. Urine-collect sue a sterile container, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again. The Operation of Hydrothorax and cerebrospinal fluid

Reference to it.

6. cell culture supernatant-detect secretory components, collect sue a sterile container, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, detect the composition of cells, Dilute cell suspension with PBS (PH7.2-7.4), Cell concentration reached 1 million / ml, repeated freeze-thaw cycles, damage cells and release of intracellular components, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.

7. Tissue samples- After cutting samples, check the weight, add PBS (PH7.2-7.4), Rapidly frozen with liquid nitrogen, maintain samples at 2-8℃ after melting, add PBS (PH7.4), Homogenized by hand or Grinders, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

Assay procedure

1. Standard dilution:

this test kit will supply one original Standard reagent, please dilute it by yourself according to the instruction.

240ng/ml	Standard No.5	120μl Original Standard + 120μl Standard diluents
120ng/ml	Standard No.4	120μl Standard No.5 + 120μl Standard diluents
60ng/ml	Standard No.3	120μl Standard No.4 + 120μl Standard diluent
30ng/ml	Standard No.2	120μl Standard No.3 + 120μl Standard diluent
15ng/ml	Standard No.1	120μl Standard No.2 + 120μl Standard diluent

2. The quantity of the plates depends on the quantities of to-be-tested samples and the standards. It is suggested to duplicate each standard and blank well. Every sample shall be made according to your required quantity, and try to use the duplicated well as possible.

3. Inject samples:

① Blank well: don’ t add samples and (CTX-Ⅱ) -antibody labeled with biotin, Streptavidin-HRP, only Chromogen solution A and B, and stop solution are allowed; other operations are the same.

② Standard wells: add standard 50μl, Streptavidin-HRP 50μl(since the standard already has combined biotin antibody, it is not necessary to add the antibody);

③ To be test wells: add sample 40μl, and then add both (CTX-Ⅱ) -antibody 10μl and Streptavidin-HRP 50μl. Then seal the sealing memberance, and gently shaking, incubated 60 minutes at 37 ℃.

4. Confection: dilute 30 times the 30×washing concentrate with distilled water as standby.

5. Washing: remove the memberance carefully, and drain the liquid, shake away the remaining water.

6. Add chromogen solution A 50μl, then chromogen solution B 50μl to each well. Gently mixed, incubate for 10 min at 37℃ away from light.

7. Stop: Add Stop Solution 50μl into each well to stop the reaction (the blue changes into yellow immediately).

8. Final measurement: Take blank well as zero, measure the optical densit (OD) under 450 nm wavelength which should be carried out within 15min after adding the stop solution.

9. According to standards’ concentration and the corresponding OD values, calculate out the standard curve linear regression equation, and then apply the OD values of the sample on the regression equation to calculate the corresponding sample’ s concentration. It is acceptable to use kinds of software to make calculations.

Summary procedures

Preparing reagents, samples and standards

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Add prepared samples and standards, antibodies labeled with enzyme, reacting 60 minutes at 37 ℃

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Plate washed five times, adding Chromogen solution A, B, reacting 10 minutes at 37℃

⇩

Add stop solution

⇩

measure the OD value within 10min

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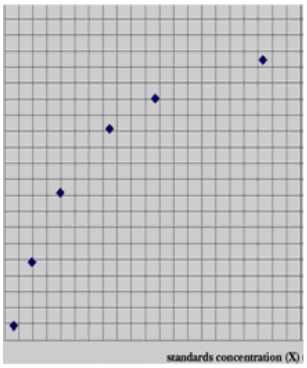
Calculation

Calculate

Take the standard density as the horizontal, the OD value for the vertical, draw the standard curve on graph paper, Find out the corresponding density according to the sample OD value by the Sample curve (the result is the sample density)



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or calculate the straight line regression equation of the standard curve with the standard density and the OD value ,with the sample OD value in the equation, calculate the sample density.

Sensitivity、Assay range

Sensitivity: **1.395ng/ml** (The sensitivity of this assay was defined as the lowest protein concentration that could be differentiated from zero. It was determined by sub-tracing two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.)

Assay range:**2ng/ml→400ng/ml**

Specificity

This assay has high sensitivity and excellent specificity for detection of CTX-Ⅱ.No significant cross-reactivity or interference between CTX-Ⅱ and analogues was observed.

※ Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between CTX-Ⅱ and all the analogues, therefore, cross reaction may still exist.

※ Sample linear regression with the expected concentration of the correlation coefficient R is over 0.95

Package size

96T per box

Validity & Storage

six months (2-8℃)