Rat cardiac isoform of Tropnin T(cTnT)ELISA Kit

【Instruction】

【Sample Types Validated】

Serum, blood plasma,Saliva, Urine, and other related tissue Liquid.

Please read this insert completely prior to using the product.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Cat.No：MBS163975

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

【Intended Use】

This kit is used to assay the cardiac isoform of Tropnin T(cTnT)in the sample of Rat'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 Rat cardiac isoform of Tropnin T（cTnT）in samples. Add cardiac isoform of Tropnin T（cTnT） to monoclonal antibody Enzyme well which is pre-coated with Rat cardiac isoform of Tropnin T（cTnT）monoclonal antibody, incubation; then, add cardiac isoform of Tropnin T（cTnT）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 Rat Substance cardiac isoform of Tropnin T（cTnT）of sample were positively correlated.

【*Materials supplied in the Test Kit*】

1	Standard(32ng/ml)	0.5ml	7	Chromogen Solution A	6ml
2	Standard diluent	3ml	8	Chromogen Solution B	6ml
3	Microelisa Stripplate	12w×8s	9	Stop Solution	6ml
4	Str- HRP-Conjugate Reagent	6ml	10	Instruction	1
5	30×wash solution	20ml	11	Closure plate membrane	2
6	Biotin-cTnT Ab	1ml	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. Beening 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.

【Precision】

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Rat cTnT were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Rat cTnT were tested on 3 different plates, 8 replicates in each plate.

CV(%) = SD/meanX100

Intra-Assay: CV<10%

Inter-Assay: CV<12%

【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 ofHydrothorax 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, Dilut 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.

【Assay procedure】

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

16ng/ml	Standard No.5	120μl Original Standard + 120μl Standard diluents
8ng/ml	Standard No.4	120μl Standard No.5 + 120μl Standard diluents
4ng/ml	Standard No.3	120μl Standard No.4 + 120μl Standard diluent
2ng/ml	Standard No.2	120μl Standard No.3 + 120μl Standard diluent
1ng/ml	Standard No.1	120μl Standard No.2 + 120μl Standard diluent

32 ng/ml 16ng/ml 8ng/ml 4ng/ml 2ng/ml 1ng/ml

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: Blank well: don't add samples and cTnT-antibody labeled with biotin, Streptavidin-HRP, only Chromogen solution A and B, and stop solution are allowed; other operations are the same. Standard wells: add standard 50µl, Streptavidin-HRP 50µl (since the standard already has combined biotin antibody, it is not necessary to add the antibody); To be test wells: add sample 40µl, and then add both cTnT-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 ℃

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Add stop solution

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measure the OD value within 10min

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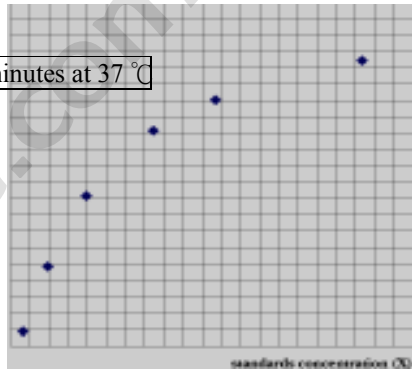
Calculation



【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)。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。

Assay range：0.1ng/ml→30ng/ml.

Sensitivity：0.06ng/ml.

Package size：96T per box.

validity&Storage：Six months(2-8℃)or Twelve months(-20℃)
[see label on the outer box for the specific date].