Version 22-04/24

User's Manual

Human NTxI(Cross Linked N-telopeptide of Type I Collagen) ELISA Kit

REF **DEIANS031**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

FOR RESEARCH USE ONLY. Not for clinical diagnosis use. The ELISA test kit provides a quantitative assay for human antibodies against NTx I in serum, plasma, Urine,tissue homogenates and cell culture supernates.

Reagents And Materials Provided

1. Assay plate (12 × 8 coated Microwells) 1
2. Standard: 22.5 nmol/L 1×0.5ml
3. Standard Diluent: 1×1.5ml
4. HRP-Conjugate Reagent: 1×6ml
5. Sample Diluent: 1×6ml
6. Chromogen Solution A: 1×6ml
7. Chromogen Solution B: 1×6ml
8. Stop Solution: 1×6ml
9. Wash Solution: 1×20ml×30 fold
10. User manual 1
11. Adhesive Strip 2

Storage

The unopened kit shall be stored at 2-8°C

For opened kit can be stored at 2-8°C for up to 1 month. If not be used recently, the standard should be kept in -20°C

Specimen Collection And Preparation

1. Serum-coagulation at room temperature for 10-20 min, centrifuge at the speed of 2000-3000 rpm for 20 min. Remove supernatant, if precipitation appeared, Centrifuge again. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
2. Plasma-use suited EDTA or citrate plasma as an anticoagulant, centrifuge at the speed of 2000-3000 rpm for 20 min. Remove supernatant, if precipitation appeared, centrifuge again. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles. Centrifuge the sample again after thawing before the assay.
3. Urine-collect sue a sterile container, centrifuge at the speed of 2000-3000 rpm for 20 min. Remove supernatant, if precipitation appeared, Centrifuge again. The Operation of Hydrothorax and cerebrospinal fluid reference to it. Assay immediately or aliquot and store samples at -20°C or -80°C.
4. Cell culture supernatant-detect secretory components, Remove particulates by centrifugation for 20 min at the speed of 2000-3000 rpm. 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 rpm. remove supernatant, If precipitation appeared, Centrifugal again. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

5. Tissue samples- After cutting samples, check the weight, Pipette PBS(PH7.2-7.4), Rapidly frozen with liquid nitrogen, maintain samples at 2-8°C after melting, Pipette PBS(PH7.4), homogenized by hand or Grinders, centrifugation 20 min at the speed of 2000-3000 rpm. Remove supernatant. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freezethaw cycles. Centrifuge the sample again after thawing before the assay.

Note:

1. Extract as soon as possible after Samples collection, and should be tested as soon as possible after the extraction. If not, samples must be stored at -20°C (≤ 1 month) or -80°C (≤ 2 months) to avoid loss of bioactivity and contamination.

2. Can' t detect the sample which contain NaN_3, because NaN_3 inhibits HRP active.

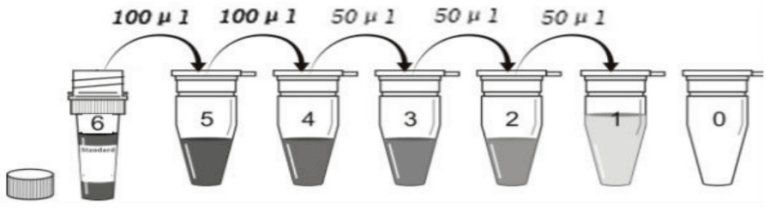
Reagent Preparation

1. Wash Buffer (1×)

If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate (30×) into deionized or distilled water to prepare 600 ml of Wash Buffer (1×).

2. Standard

Dilute the standard: Pipette 50µl standard diluent in each tube. Pipette 100µl standard (22.5 nmol/L) in the fifth tube. And take out 100µl from the fifth five tube into the fourth. Pipette 50µl from the fourth tube to the third tube and produce dilution series as below. The undiluted Standard serves as the high standard (22.5 nmol/L). Sample Diluent serves as the zero standard(blank well)(0 nmol/L).



Tube	6	5	4	3	2	1	0
nmol/L	22.5	15	10	5	2.5	1.25	0

Assay Procedure

Washing Notes

1. Manually washing method: shake away the remained 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.

2. 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.

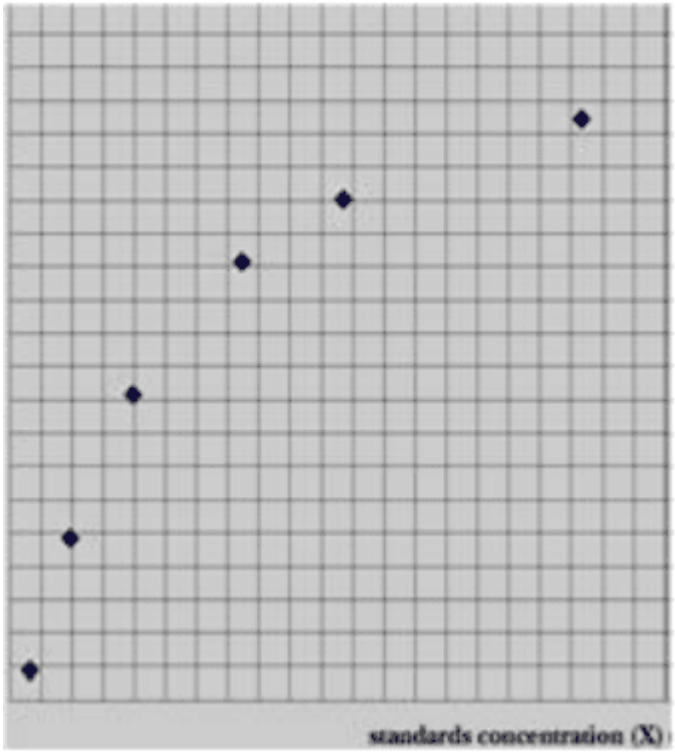
Procedure

1. Prepare all reagents, working standards, Blank and samples as directed in the previous sections.
2. Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Pipette standard 50µl to testing standard well,Pipette Sample diluent 40µl to testing sample well, then add testing sample 10µl (sample final dilution is 5-fold), Pipette sample to wells, don' t touch the well wall as far as possible, and mix gently.
4. Incubate: Cover with the adhesive strip provided, incubate for 30 min at 37°C.
5. Configurate liquid: Dilute wash solution 30-fold with distilled water.
6. Washing: Uncover the adhesive strip, discard liquid, pipette washing buffer to every well, still for 30s then drain, repeat 5 times.
7. Add enzyme: Pipette HRP-Conjugate reagent 50µl to each well, except blank well.
8. Incubate: Operation with 4
9. Washing: Operation with 6.
10. Color: Pipette Chromogen Solution A 50µl and Chromogen Solution B 50µl to each well, avoid the light reservation for 15 min at 37°C.
11. Stop the reaction: Pipette Stop Solution 50µl to each well, stop the reaction (the blue change to yellow).
12. Calculate: take blank well as zero. Read absorbance at 450 nm after pipette Stop Solution within 15 min.

Calculation

Take the standard concentration as the horizontal, the OD value for the vertical ,draw the standard curve on graph paper, Find out the corresponding concentration according to the sample OD value by the Sample curve, multiplied by the dilution multiple, or calculate the straight line regression equation of the standard curve with the standard concentration and the OD value, with the sample OD value in the equation, calculate the sample concentration, multiplied by the dilution factor, the result is the sample actual concentration.

Typical Standard Curve



Precision

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Detection Range

0.7nmol/L-20 nmol/L

Sensitivity

0.1nmol/L

Precautions

1. The kit takes out from the refrigeration should be balanced 15-30 minutes in the room temperature, if the coated ELISA plates have not been used up after opening, the plate should be stored in sealed bag.
2. Washing buffer will Crystallization separation, it can be heated in water to dissolve.
3. Pipette sample with pipettors each step, and proofread its accuracy frequently to avoid the experimental error. Pipette sample within 5 min, if the number of sample is big, recommend using multichannel pipettor.
4. If the testing material concentration is excessively high (The sample OD is higher than the first standard well),please dilute the sample (n-fold).
5. Adhesive Strip only limits the disposable use to avoid cross-contamination.

6. The substrate should evade the light to be preserved.
7. Please refer to the user instruction strictly, the test result determination must take the microtiter plate reader as a standard.
8. The preparation of samples and all the reagents should refer to infective material process.
9. Do not mix reagents with those from other lots.