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BTB-E1239Hu 96Tests

Enzyme-linked Immunosorbent Assay Kit

Human N-terminal pro-brain natriuretic peptide(NT-PROBNP)ELISA Kit

Instruction Manual

【INTENDED USE】

The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of NT-PROBNP in human's serum, tissue homogenates and other biological fluids.

【TEST PRINCIPLE】

This kit uses enzyme-linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technology to assay the Human N-terminal pro-brain natriuretic peptide (NT-PROBNP). Add N-terminal pro-brain natriuretic peptide(NT-PROBNP)to the wells, which are pre-coated with N-terminal pro-brain natriuretic peptide(NT-PROBNP)monoclonal antibody and then incubate. After that, add anti NT-PROBNP antibodies labeled with biotin to unite with streptavidin-HRP, which forms immune complex. Remove unbound enzymes after incubation and washing. Add substrate A and B. Then the solution will turn blue and change into yellow with the effect of acid. The shades of solution and the concentration of Human N-terminal pro-brain natriuretic peptide (NT-PROBNP) are positively correlated.

【MATERIALS REQUIRED BUT NOT SUPPLIED】

1. An incubator which can provide stable incubation conditions up to $37^{\circ}\text{C}\pm 0.5^{\circ}\text{C}$.
2. Microplate reader with $450 \pm 10\text{nm}$ filter.
3. Precision pipettes and Disposable pipette tips.
4. Distilled water.
5. Deionized or distilled water.
6. Absorbent paper.

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【MATERIALS SUPPLIED IN THE TEST KIT】

Reagents	Quantity	Storage
Standard solution(2000ng/L)	0.5ml	May be stored for up to 1 month at 2-8° C. If don't make recent use, better keep it store at -20°C.
Coated ELISA plate	12-Well * 8 Tubes	
Standard dilution	3ml	
Streptavidin-HRP	6ml	
Stop solution	6ml	
Chromogen solution A	6ml	
Chromogen solution B	6ml	
Washing concentrate (30X)	20ml	
Anti NT-PROBNP antibodies labeled with biotin	1ml	
Instruction	1	
Seal plate membrane	2	
Hermetic bag	1	

【IMPORTANT NOTES】

1. Before opening the kit kept at the temperature of 2-8□, it takes at least 30 minutes to increase naturally to room temperature. After breaking the seal of ELISA coated-plate, some of the stripes used should be kept in hermetic bag.
2. When adding samples, sample injector must be used for each time and should also be frequently checked for its precision to avoid individual error.
3. The instruction must be strictly followed while the reading of ELISA reader must be set as the standard of determining the experiment result.
4. Pipette tips and seal plate membrane in hand should not be used more than once in order to avoid cross contamination.
5. All samples, washing concentration and wastes of every kind should be disposed as infective agent.
6. Other reagents not needed must be packed or covered. Reagents of different batches must not be mixed and should be used before their respective validity dates.

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7. Substrate B is sensitive to light and therefore should not be exposed to light for too long.

【WASHING METHOD】

Manually washing method: Washing by hand: Shake off the liquids in the wells of the ELISA plate; Lay several bibulous papers on the test bed and pat hard the ELISA plate several times downward; then inject at least 0.35ml of diluted washing concentration for 1-2 minutes' soaking. Repeat this process as needed.

Automatic washing method: Washing by automatic plate washer: If there is an automatic plate washer, it should only be used in the test when you are quite familiar with its functions.

【PRECISION】

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

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

$$CV(\%) = SD/\text{mean} \times 100$$

Intra-Assay: CV<8%

Inter-Assay: CV<10%

【SPECIMEN REQUIREMENTS】

1. Samples containing NaN₃ must not be tested as it inhibits the activity of Horse Radish Peroxidase (HRP).

2. After collecting the sample, extraction should be immediately carried out in accordance with related documents. After extraction, experiment should be conducted immediately as well. Otherwise, keep the sample at -20℃. Avoid repeated freeze-thaw cycles.

3. Serum: Allow the serum to clot for 10-20 minutes at room temperature. Centrifuge (at 2000-3000

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RPM) for 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.

4. Blood plasma: In accordance with the requirements of sample collection, EDTA or sodium citrate should be used as anti coagulation. Add EDTA or sodium citrate and mix them for 10-20 minutes. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.

5. Urine: Collect by sterile tube. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again. When collecting pleuroperitoneal fluid and cerebrospinal fluid, please follow the procedures above-mentioned.

6. Cell culture supernatant: Collect by sterile tubes when examining secrete components. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. When examining the components within the cell, use PBS (PH 7.2-7.4) to dilute cell suspension to the cell concentration of approximately 1 million/ml. Damage cells through repeated freeze-thaw cycles to let out the inside components. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.

7. Tissue sample: Incise sample and weigh up. Add a certain amount of PBS (PH 7.4). Freeze with liquid nitrogen immediately for later use. Thaw the sample and keep it at 2-8°C. Add a certain amount of PBS (PH 7.4) and then homogenize the sample thoroughly by hand or homogenizer. Centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully. Aliquot and keep one for examination and freeze the others for later use.

Note:

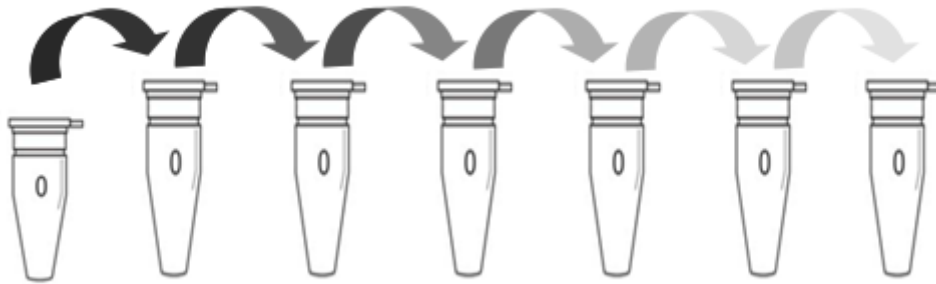
1. Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be detected.
3. When performing the assay, bring samples to room temperature.

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【ASSAY PROCEDURE】

1. Dilution of standard solutions: (This kit has a standard of original concentration, which could be diluted in small tubes by user independently following the instruction.):

1000ng/L	Standard No.5	120μl Original Standard + 120μl Standard diluents
500ng/L	Standard No.4	120μl Standard No.5 + 120μl Standard diluents
250ng/L	Standard No.3	120μl Standard No.4 + 120μl Standard diluent
125ng/L	Standard No.2	120μl Standard No.3 + 120μl Standard diluent
62.5ng/L	Standard No.1	120μl Standard No.2 + 120μl Standard diluent



STANDARD	S5	S4	S3	S2	S1
2000ng/L	1000ng/L	500ng/L	250ng/L	125ng/L	62.5ng/L

2. The number of stripes needed is determined by that of samples to be tested added by that of standards. It is suggested that each standard solution and each blank well should be arranged with three or more wells as much as possible.

3. Sample injection: 1) Blank well: no sample, anti NT-PROBNP antibody labeled with biotin or streptavidin-HRP is added to comparison blank well except chromogen solution A & B and stop solution while taking the same steps that follow. 2) Standard solution well: Add 50μl standard and streptavidin-HRP 50μl (biotin antibodies have united in advance in the standard so no biotin antibodies are added.) 3) Sample well to be tested: Add 40μl sample and then 10μl NT-PROBNP antibodies, 50μl streptavidin-HRP. Then cover it with seal plate membrane. Shake gently to mix them up. Incubate at 37°C for 60 minutes.

4. Preparation of washing solution: Dilute the washing concentration (30X) with distilled water for

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later use.

5. Washing: Remove the seal plate membrane carefully, drain the liquid and shake off the remaining liquid. Fill each well with washing solution. Drain the liquid after 30 seconds' standing. Then repeat this procedure five times and blot the plate.

6. Color development: Add 50µl chromogen solution A firstly to each well and then add 50µl chromogen solution B to each well as well. Shake gently to mix them up. Incubate for 10 minutes at 37°C away from light for color development.

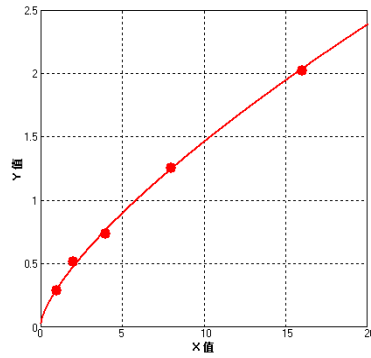
7. Stop: Add 50µl Stop Solution to each well to stop the reaction (the blue color changes into yellow immediately at that moment).

8. Assay: Take blank well as zero, measure the absorbance (OD) of each well one by one under 450nm wavelength, which should be carried out within the 10 minutes after having added the stop solution.

9. According to standards' concentrations and the corresponding OD values, calculate the linear regression equation of the standard curve. Then according to the OD value of samples, calculate the concentration of the corresponding sample. Special software could be employed to calculate as well.

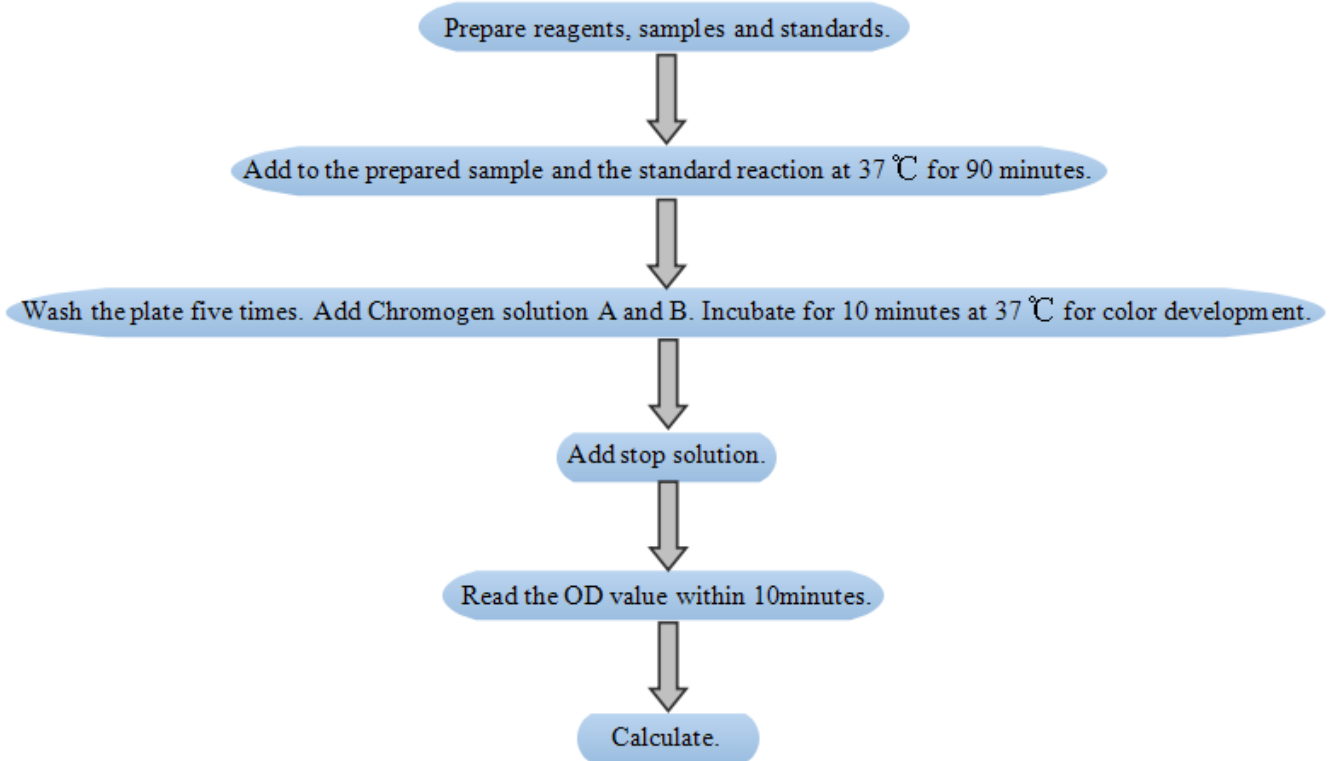
【CALCULATE】

Make concentration of standards the abscissa and OD value the ordinate. Draw the standard curve on the coordinate paper. According to the OD value of the sample, locate its corresponding concentration (which is the concentration of the sample); or calculate the linear regression equation of standard curve according to the concentration of the standard and the OD value. Then substitute with the OD value of the sample to calculate its concentration.



[THE CHART IS FOR REFERENCE ONLY AND THE ACTUAL PERFORMANCE DON'T HAVE TO BE THE SAME AS THE CHART]

【SUMMARY】



ASSAY RANGE : 5ng/L→2000ng/L.

SENSITIVITY : 2.49ng/L.

VALIDITY&STORAGE : Six months(2-8℃)or Twelve months(-20℃).See label on the outer box for the specific date.

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