Human NT-proBNP ELISA Kit

Catalog No.: abx050180

Size: 96T

Range: 62.5 pg/ml - 4000 pg/ml

Sensitivity: 2 pg/ml

Storage: Store at 4°C for 6 months or at -20℃ for one year. Store HRP conjugated antibody at 4℃ for one year.

Application: For quantitative detection of NT-proBNP in Human Serum, Cell Culture Supernatants and plasma.

Introduction: The N-terminal prohormone of brain natriuretic peptide (NT-proBNP or BNPT) is a prohormone with a 76 amino acid N-terminal inactive protein that is cleaved from the molecule to release brain natriuretic peptide. Both BNP and NT-proBNP levels in the blood are used for screening, diagnosis of acute congestive heart failure (CHF) and may be useful to establish prognosis in heart failure, as both markers are typically higher in patients with worse outcome. The plasma concentrations of both BNP and NT-proBNP are also typically increased in patients with asymptomatic or symptomatic left ventricular dysfunction and is associated with coronary artery disease and myocardial ischemia.

Principle of the Assay

This kit is based on sandwich enzyme-linked immune-sorbent assay technology. Anti-NT-proBNP polyclonal antibody is pre-coated onto 96-well plates. The standards and test samples are added to the wells and any NT-proBNP present is bound by the immobilized antibody. Unbound conjugates are washed away and the HRP conjugated anti-NT-proBNP monoclonal antibody is added to the wells as a detection antibody. TMB substrate is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the NT-proBNP amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450nm in a microplate reader, and then the concentration of NT-proBNP can be calculated.

Kit components

1. One 96 well plate.
2. Standard: 2 tubes
3. Sample / Standard diluent buffer: 30ml
4. HRP conjugated antibody: 130 µl - Dilution: 1:100 (DO NOT FREEZE!)
5. Antibody diluent buffer: 12 ml
6. TMB substrate: 10 ml
7. Stop solution: 10 ml
8. Wash buffer (25X): 30 ml

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. Multi and single channel pipettes and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. ELISA shaker
6. 1.5 ml tubes to prepare standard/sample dilutions
7. Deionized or distilled water
8. Absorbent filter papers
9. 100 ml and 1 liter graduated cylinders

Protocol

A. Preparation of sample and reagents

1. Sample

Store samples to be assayed within 2 hours of collection. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum at room temperature (~2 hr) or overnight at 4°C. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Cell culture supernatant:** Centrifuge to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using EDTA or heparin as the anticoagulant. Centrifuge for 20 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Citrate can not be used as anticoagulant.

Note:

» Samples must be diluted so that the expected concentration falls within the kit's range.
» Fresh samples or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results.
» Please bring sample slowly to room temperature. Coagulate blood samples completely before centrifugation. Avoid hemolysis and particle. Grossly hemolyzed or lipemic samples may not be suitable.
» NaN3 cannot be used as test sample preservative, since it inhibits HRP.

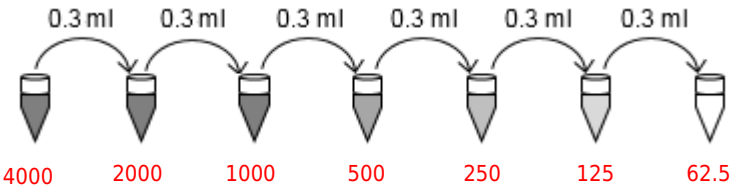
2. Wash buffer

Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 30 ml of concentrated wash buffer into 720 ml of distilled water).

3. Standard

The standard solution should be prepared no more than 2 hours prior to the experiment. Note: Do not dilute the standard directly in the plate. Two tubes of standard are included in each kit. Use one tube for each experiment.
a.) 10000 pg/ml of standard solution: Add 1 ml of Sample/Standard diluent buffer into one Standard tube to make the 10000 pg/ml solution. Keep the tube at room temperature for 10 min, mix thoroughly and avoid foaming or bubbles. Further dilute the above solution by a factor of 2.5 to give the highest standard (4000 pg/ml).
b.) 4000 pg/ml → 62.5 pg/ml standard solutions: Label 7 tubes with 4000 pg/ml, 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, and 62.5 pg/ml. Aliquot 0.3 ml of the Sample / Standard diluent buffer into each tube. Add 0.3 ml of the above 4000 pg/ml standard solution into the 1st tube and mix thoroughly. Transfer 0.3 ml from the 1st tube to the 2nd tube and mix thoroughly. Transfer 0.3 ml from the 2nd tube to the 3rd tube and mix thoroughly, and so on.

10,000 pg/ml
NT-proBNP Solution



Note: The standard solutions are best used within 2 hours. The standard solution should be used within 12 hours. Or store at -20 °C for up to 48 hours. Avoid repeated freeze-thaw cycles.

4. Preparation of HRP working solution: prepare no more than 30 min. before the experiment.
a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).
b.) Dilute the HRP conjugated antibody with Antibody diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of HRP conjugated antibody into 99 µl of Antibody diluent buffer.

B. Assay Procedure
Equilibrate all kit components to room temperature before use.

1. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommend to measure each sample, standard, zero and optional control sample in duplicate or triplicate. Add the solution at the bottom of each well without touching the side walls. Store the unused strips back in the foil bag at 4℃, or at -20℃ for long term.
2. Aliquot 0.1ml of the diluted standards into the standard wells.
3. Add 0.1 ml of Sample / Standard diluent buffer into the control (zero) well.
4. Add 0.1 ml of appropriately diluted sample into the test sample wells.
5. Seal the plate with a cover and incubate at 37℃ for 90 min.
6. Remove the cover and discard the solution. Wash the plate 5 times with 1X Wash Buffer. Fill each well completely with Wash buffer (300µL) using a multi-channel Pipette or autowasher (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
7. Add 0.1 ml of HRP conjugated antibody working solution into the wells **(except control / zero well).** Add the solution at the bottom of each well without touching the side wall.
8. Seal the plate with a cover and incubate at 37℃ for 60 min.
9. Remove the cover and wash the plate 5 times with Wash buffer and each time let the wash buffer stay in the wells for 1-2 min. (Repeat Step 6).
10. Add 0.1 ml of TMB substrate into each well, cover the plate and incubate at 37℃ for 25-30 min. Incubation time is for reference only. Different shades of blue should be observed in the first 3-4 wells (with most concentrated Human NT-proBNP standard solutions). Other wells will show no obvious color. (Note: The color development on the plate should be monitored and the substrate reaction stopped before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay. It is recommended to add the stop solution when the highest standard has developed a dark blue color.)
11. Add 0.1 ml of Stop solution into each well to stop the enzyme reaction. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
12. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450nm immediately.

For calculation: (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human NT-proBNP concentration of the samples can be interpolated from the standard curve.
Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution. Calculation of samples with a concentration exceeding 2000pg/ml may result in incorrect, low human NT-proBNP levels. Such samples require further external pre-dilution according to expected human NT-proBNP values with Sample / Standard diluent buffer in order to precisely quantitate the actual human NT-proBNP level.

C. Precautions

1. Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid.
2. If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. Avoid foaming or bubbles when mixing or reconstituting components. Prepare the Standard dilutions within 15 min of use and discard any unused working standards. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
4. Do not let the wells uncovered for extended periods between incubation. Once reagents are added to the wells, avoid letting the strips dry as this can inactivate the biological material on the plate. Incubation time and temperature must be controlled.
5. Ensure plates are properly sealed or covered during incubation steps.
6. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
7. Do not reuse pipette tips and tubes to avoid cross contamination.
8. Do not vortex the standard during reconstitution, as this will destabilize the protein. Once your standard has been reconstituted, it should be used right away. We do not recommend reusing the reconstituted standard.
9. The TMB Substrate solution is easily contaminated; work under sterile conditions when handling the TMB substrate solution. The TMB Substrate solution should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.
10. Store HRP conjugated antibody at 4℃, do NOT store it at -20℃.