



User's Manual

# Human NT-proBNP ELISA

For detection of NT-proBNP in human serum, plasma,  
and cell culture supernates.



**Storage:**

**BE69057**

**96**

**2-8°C**

**RUO**

**For Research Use Only – Not for Use in Diagnostic Procedures.**

## 1 INTRODUCTION

### 1.1 Intended Use

For detection of NT-proBNP in human serum, plasma and cell culture supernates. For research use only, not for use in diagnostic procedures.

### 1.2 Background

The N-terminal prohormone of brain natriuretic peptide (NT-proBNP) which consists of 76 amino acids, is the N-terminal fragment of 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. BNP may be a useful screening tool for left ventricular dysfunction in patients with history suggestive of heart disease and be used to assist in forming a pretest probability, which in turn could greatly assist in appropriateness of patient referral and in optimization of drug therapy.

## 2 PRINCIPLE OF THE TEST

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Anti-NT-proBNP polyclonal antibody was pre-coated onto 96-well plates. The standards and test samples were added to the wells and any NT-proBNP present is bound by the immobilized antibody. After washing away any unbound substances, the HRP conjugated anti-NT-proBNP monoclonal antibody was added to wells as detection antibodies. Following a wash to remove any unbound antibody-enzyme reagent, the TMB substrates were added to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding stop solution. The density of yellow is proportional to the NT-proBNP amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of NT-proBNP can be calculated.

## 3 WARNINGS AND PRECAUTIONS

1. This kit is for research use only, not for use in diagnostic procedures.
2. All reagents should be considered as potentially hazardous. It is recommended that this kit is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
3. Store HRP conjugated anti-human NT pro-BNP antibody (concentrated) (Kit Component 4) at 4°C, do NOT store at -20°C.
4. Do not use expired components or mix components from different lots or suppliers.
5. Avoid contact of skin or mucous membranes with kit reagents or specimens.
6. Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
7. Avoid contact of substrate solution with oxidizing agents and metal.
8. Avoid splashing or generation of aerosols.
9. To avoid microbial contamination or cross-contamination of reagents or specimens, it is recommended to use the clean and separate pipette tip for each.
10. Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent. Exposure to acid inactivates the conjugate.
11. Distilled or deionized water must be used for reagent preparation.
12. Substrate solution must be equilibrated at room temperature prior to use.

## 4 REAGENTS

### 4.1 Reagents provided

1. One 96-well plate pre-coated with anti-human NT-proBNP antibody
2. Human NT-proBNP standards: 2 tubes (10 ng / tube)
3. Sample / Standard diluent buffer: 30ml
4. HRP conjugated anti-human NT-proBNP antibody (Concentrated): 130µl. Dilution: 1:100 (Store at 4°C, do NOT store at -20°C)
5. Antibody diluent buffer: 12ml
6. TMB substrate: 10ml
7. Stop solution: 10ml
8. Wash buffer: 30ml (25x). Dilution: 1:25

**Note: Reconstitute standards and test samples with Kit Component 3.**

## 4.2 Materials required but not provided

1. Microplate reader (wavelength: 450nm)
2. Precise pipette and disposable pipette tips
3. Deionized or distilled water
4. Automated plate washer
5. Beakers, flasks or cylinders, etc.
6. Statistical calculator
7. Plate cover
8. Absorbent filter papers or absorbent material
9. ELISA shaker

## 4.3 Storage Conditions / Expiration

Store at 4°C for 6 month, or at -20°C for one year. Store HRP conjugated antibody at 4°C for one year.

## 4.4 Preparation of sample and reagents

The concentrated reagents should be brought to room temperature and should be diluted before starting the test procedure. If crystals have formed in the reagents, warm them gently until they have completely dissolved.

### 1. Sample

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

✧ **Cell culture supernatants:** Centrifuge to remove particulates, analyze immediately or aliquot and store at -20°C. Avoid multiple freeze-thaw cycles.

✧ **Serum:** Coagulate the serum at room temperature (about 2 hours). Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C. Avoid multiple freeze-thaw cycles.

✧ **Plasma:** Collect plasma with EDTA or heparin as the anticoagulant. Centrifuge for 20 min at 1000 × g within 30 min of collection. Analyze immediately or aliquot and store at -20°C. Avoid multiple freeze-thaw cycles. **Citrate cannot be used as anticoagulant here.**

**Note:** 1. Coagulate blood samples completely, then, centrifuge, and avoid hemolysis and particle. Grossly hemolyzed or lipemic samples may not be suitable for measurement of human NT-proBNP with this assay.

2. NaN<sub>3</sub> can not be used as test sample preservative, since it is the inhibitor for HRP.

### 2. Wash buffer

Pour entire contents (30 ml) of the Wash buffer (25x) (Kit Component 8) into a clean 750 ml graduated cylinder. Bring to final volume of 750 ml with distilled or deionized water. Mix gently to avoid foaming.

Then, transfer to a clean wash bottle and store at 2-25°C. Wash buffer (1x) can be stable for 30 days.

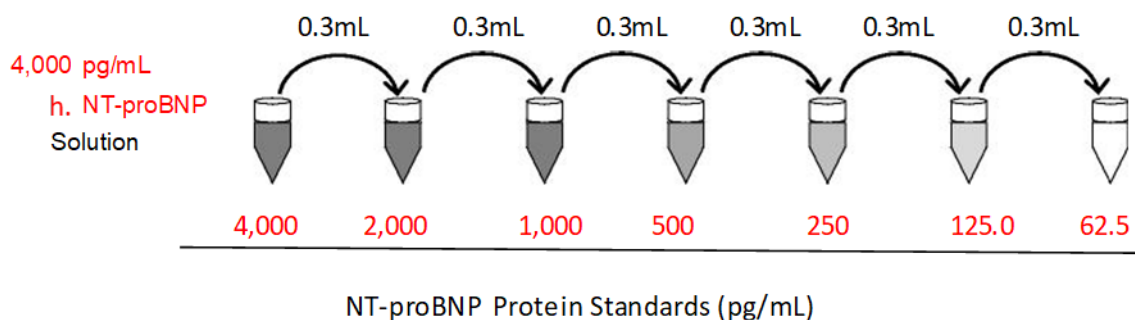
### 3. Standard

Reconstitution of the lyophilized human NT-proBNP standard (Kit Component 2): standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of standard are included in each kit. Use one tube for each experiment. (**Note: Do not dilute the standard directly in the plate**)

a. 10,000 pg/ml of standard solution: Add **1 ml** of Sample / Standard diluent buffer (Kit Component 3) into one Standard (Kit Component 2) tube, keep the tube at room temperature for 10 min and mix thoroughly.

b. 4000 pg/ml of standard solution: Add **0.4 ml** of the above 10 ng/ml standard solution into **0.6 ml** sample diluent buffer (Kit Component 3) and mix thoroughly.

c. 2000 pg/ml → 62.5 pg/ml of standard solutions: Label 6 Eppendorf tubes with 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, respectively. Aliquot **0.3 ml** of the Sample / Standard diluent buffer (Kit Component 3) into each tube. Add **0.3 ml** of the above 2000 pg/ml standard solution into 1st tube and mix thoroughly. Transfer **0.3 ml** from 1st tube to 2nd tube and mix thoroughly. Transfer **0.3 ml** from 2nd tube to 3rd tube and mix thoroughly, and so on.



**Note:** The standard solutions are best used within 2 hours. The 4000 pg/ml 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 conjugated anti-human NT-proBNP antibody (Kit Component 4) working solution: the HRP conjugated antibody should be used within 30 min after diluting.

- Calculate the total volume of the working solution: 0.1 ml / well x quantity of wells. (Allow 0.1-0.2 ml more than the total volume)
- Dilute the HRP conjugated anti-human NT-proBNP antibody (Kit Component 4) with Antibody diluent buffer (Kit Component 5) at 1:100 and mix thoroughly. i.e. Add 1 µl of HRP conjugated anti-human NT-proBNP antibody into 99 µl of Antibody diluent buffer.

## 5 ASSAY PROCEDURE

### 5.1 General Remarks

It is required to plot a standard curve for each test.

### 5.2 Test Procedure

Equilibrate all components to room temperature before use.

- Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommended to measure each sample, standard, zero and optional control sample in duplicate. Remove extra microwell strips from holder and store them in foil bag at 4°C, or at -20°C for long term.
- Aliquot 0.1ml of 4000 pg/ml, 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml standard solutions into the standard wells.
- Add 0.1 ml of Sample / Standard diluent buffer (Kit Component 3) into the control (zero) well.
- Add 0.1 ml of properly diluted sample (human serum, plasma or cell culture supernatants) into test sample wells.

**Here is an example of the arrangement of sample, standard and zero in the plate wells:**

	1	2	3	4
A	Standard 1 (4000.0 pg/ml)	Standard 1 (4000.0 pg/ml)	Sample 1	Sample 1
B	Standard 2 (2000.0 pg/ml)	Standard 2 (2000.0 pg/ml)	Sample 2	Sample 2
C	Standard 3 (1000.0 pg/ml)	Standard 3 (1000.0 pg/ml)	Sample 3	Sample 3
D	Standard 4 (500.0 pg/ml)	Standard 4 (500.0 pg/ml)	Sample 4	Sample 4
E	Standard 5 (250.0 pg/ml)	Standard 5 (250.0 pg/ml)	Sample 5	Sample 5
F	Standard 6 (125 pg/ml)	Standard 6 (125 pg/ml)	Sample 6	Sample 6
G	Standard 7 (62.5 pg/ml)	Standard 7 (62.5 pg/ml)	Sample 7	Sample 7
H	Zero / Blank	Zero / Blank	Sample 8	Sample 8

- Seal the plate with a cover and incubate at 37°C for 90 min.
- Remove the cover, aspirate the plate content and wash plate 5 times with Wash buffer (Kit Component 8) using one of the following methods:

**Manual Washing:** Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with Wash buffer (Kit Component 8) and vortex mildly on ELISA shaker for 2 min, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure four more times for a **total of FIVE washes**.

**Automated Washing:** Aspirate all wells, then wash plate **FIVE times** with Wash buffer (Kit Component 8) (overfilling wells with the buffer, about 400µl). After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min or shaking.

- Add 0.1 ml of HRP conjugated anti-human NT-proBNP antibody work solution into each well (except control/zero well). Add the solution at the bottom of each well without touching the side wall.
- Seal the plate with a new cover and incubate at 37°C for 60 min.
- Remove the cover, aspirate the plate content and wash plate 5 times with Wash buffer (Kit Component 8), and each time let the wash buffer stay in the wells for 1-2 min. (Repeat Step 6).
- Add 0.1 ml of TMB substrate (Kit Component 6) into each well, cover the plate and incubate at 37°C in dark for about 30 min. (**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.) And the shades of blue can be seen in the first 3-4 wells (with most concentrated human NT-proBNP standard solutions), the other wells show no obvious color.
- Add 0.1 ml of Stop solution (Kit Component 7) into each well and mix thoroughly. The color changes into yellow immediately.
- Read the O.D. absorbance at 450 nm in a microplate reader within 30 min after adding the stop solution.  
**Note:** If the incubation without shaking, the obtained O.D. Values may be lower than the typical data, but the results are still valid.

### 5.3 Results

For calculation, average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. (The relative O.D.<sub>450</sub>) = (the O.D.<sub>450</sub> of each well) – (the O.D.<sub>450</sub> of Zero well). The standard curve can be plotted as the relative O.D.<sub>450</sub> 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:** 1. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

2. 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 (Kit Component 3) in order to precisely quantitate the actual human NT-proBNP level.

## 6 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. It is recommended to use controls according to state and federal regulations. The use of controls is advised to assure the day to day validity of results. It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results. Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials, results of unknowns should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact your distributor or IBL-America directly.

## 7 PERFORMANCE CHARACTERISTICS

### 7.1 Range

62.5pg/ml-4000pg/ml

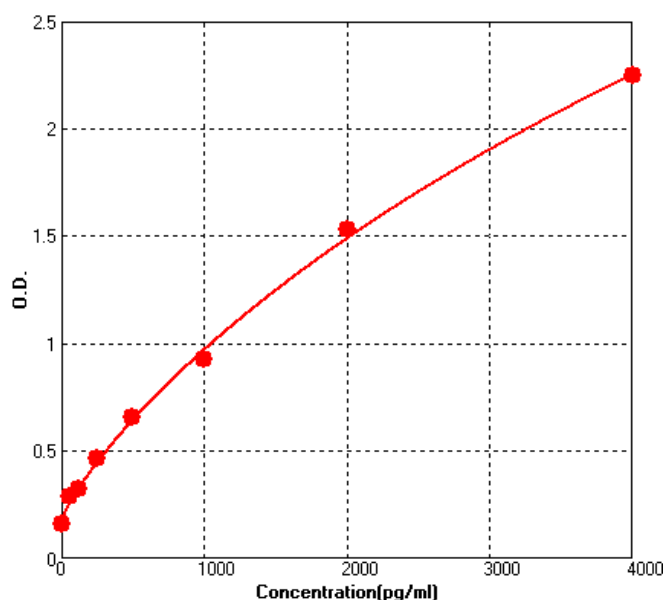
### 7.2 Sensitivity

2pg/ml

### 7.3 Typical Data & Standard Curve

Results of a typical standard run of a Human NT-proBNP ELISA Kit are shown below. **This standard curve was generated at our lab for demonstration purpose only.** Each user should obtain their own standard curve as per experiment. (N/A=not applicable)

X	pg/ml	0	62.5	125	250	500	1000	2000	4000
Y	OD450	0.163	0.289	0.325	0.464	0.660	0.930	1.527	2.248



#### **7.4 References:**

1. Bhalla V, Willis S, Maisel AS (2004). "B-type natriuretic peptide: the level and the drug--partners in the diagnosis of congestive heart failure". *Congest Heart Fail* 10 (1 Suppl 1): 3–27.
2. Atisha D, Bhalla MA, Morrison LK, Felicio L, Clopton P, Gardetto N, Kazanegra R, Chiu A, Maisel AS (September 2004). "A prospective study in search of an optimal B-natriuretic peptide level to screen patients for cardiac dysfunction". *Am. Heart J.* 148 (3): 518–23.

#### **8 ORDERING INFORMATION**

This kit is manufactured for Immuno-Biological Laboratories, Inc. (IBL-America). For ordering information, please contact:

**Immuno-Biological Laboratories, Inc. (IBL-America)**

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