For the quantitation of human NT-proBNP concentrations in cell culture supernatants, serum, and plasma.

INTRODUCTION

NT-proBNP (N-terminal pro-B-type natriuretic peptide) is an inactive amino-terminal fragment of the prohormone BNP (brain natriuretic peptide) expressed by ventricular myocytes. NT-proBNP is a 1-76 amino acid fragment that is co-released with the biologically active 32 amino acids BNP after cleavage from the prohormone. NT-proBNP is a stable and reliable biomarker for the diagnosis and management of heart failure. Elevated levels of NT-proBNP are associated with increased mortality in patients with heart failure and can be used to guide therapy and predict prognosis.

The Fast Human NT-proBNP ELISA is a solid phase ELISA designed to measure human NT-proBNP levels in cell culture supernatants, serum, and plasma. The main feature is that the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate and fast. The measurement can be finished in 1 hour, with no need for 5-6 hours (Fig. 1). The detection range is from 31 to 2000pg/mL. The levels of human NT-proBNP samples are parallel to the standard curves obtained using the kit standards linearly. These results indicate that this kit can be used to determine relative mass values for natural human NT-proBNP protein.

PRINCIPLE OF THE ASSAY

This assay employs our novel proprietary sandwich enzyme immunoassay techniques (see Fig. 1). A monoclonal antibody specific for human NT-proBNP is pre-coated onto a microplate. Standards or samples and a biotin conjugated detection antibody are pipetted into the wells and concurrently incubated to form a sandwich complex in one step. Simply aspirate each well without washing and directly add Streptavidin-HRP into the complex. Following a wash, an **ultra-sensitive TMB substrate solution** is added to the wells for color development. The color intensity is proportional to the amount of NT-proBNP bound in the initial step. The intensity of the color is measured by plate reading at 450 nm.

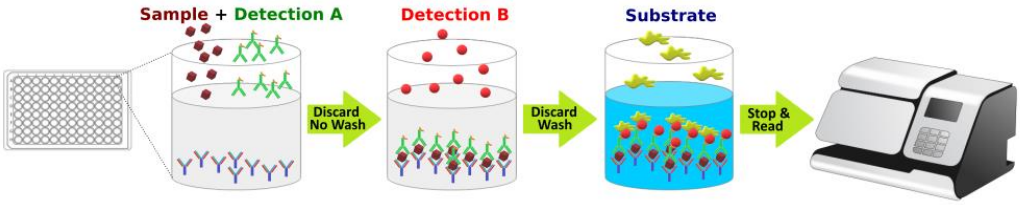


Fig.1: Simple ELISA procedure

KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Human NT-proBNP Microplate	TBS3287A	96 well microplate (12 strips of 8 wells) coated with a Capture Antibody specific for human NT-proBNP.	The unused wells can be stored in the sealed foil pouch containing the desiccant pack for up to 1 month at 2-8 °C.
Human NT-proBNP Standard	TBS3287B	30 µL of Recombinant human NT-proBNP protein (100ng/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3287C	2.1 mL of human NT-proBNP antibody.	May be stored for up to 3 months at 2-8 °C.
Detection B	TBS3287D	12 mL of Streptavidin-HRP	
Assay Diluent	TBS3287E	12 mL of a buffered protein base with preservatives.	
10x Wash Buffer	TBS3000W	12 mL of concentrated solution (10x).	
TMB Substrate	TBS3000T	12 mL of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 mL of 2 N sulfuric acid.	

Store the unopened kit at 2-8 ℃. Do not use past kit expiration date.
The kit contains sufficient materials to run an ELISA on one 96 well plate.

PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

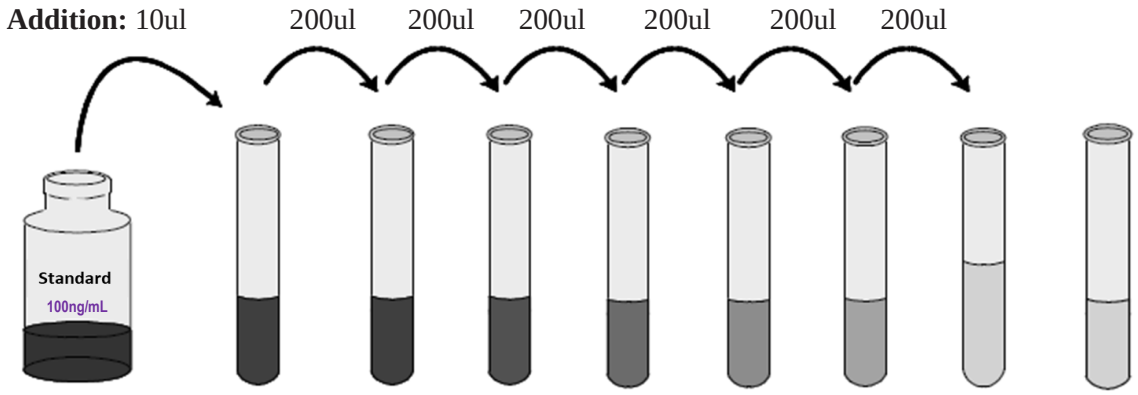
Bring all reagents to room temperature before use.

Wash Buffer: Add 10 mL of Wash Buffer Concentrate (10x) to 90 mL of deionized distilled water to prepare 100 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.*).

Human NT-proBNP Standard Preparation:

1. Label test tubes as #1 through #8. Pipet 490 µL of 1x Assay Diluent into tube #1, and 300 µL into tubes #2 to #8 **as diagram below.**
2. Add 10 µL of the Human NT-proBNP Standard stock solution (100ng/mL) by dilution of 50X to tube #1 and mix.
3. Make 2x serial dilutions of the standard using the 2000pg/mL standard solution from tube #2 through #7 with sequential transfer of 200 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 2000, 1000, 500, 250, 125, 62.5 and 31.3 pg/mL. Tube# 8 is Standard 0.

Fig.2: Diagram for Human NT-proBNP standard preparation



Standard	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	490	300	300	300	300	300	300	300
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol (µL)	10	200	200	200	200	200	200	0
Final Conc (pg/ml)	2000	1000	500	250	125	62.5	31.3	0

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Add 80 µL of standard, sample, or control per well.
2. Add 20 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 60 min.**
3. Aspirate each well (***no wash***). Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **Detection B** to each well. Incubate at **RT for 30 min.**
5. Aspirate each well, and wash for 3 times by filling each well with 300 µL Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Protect from light*). The color becomes blue. If the color is light, the incubation time can be longer.

7. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing). Read plate in 5 minutes after adding Stod Solution.
8. Determine the optical density of each well within 5 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human NT-proBNP concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

TYPICAL DATA

This standard curve (R^2=1.000) is provided in Fig.3 for demonstration only. A standard curve should be generated for each set of samples assayed.

SENSITIVITY

The minimum detectable dose (MOD) of human NT-proBNP is typically 5 pg/ml.
The Intra-CV is < 10%, the Inter-CV is < 12%.

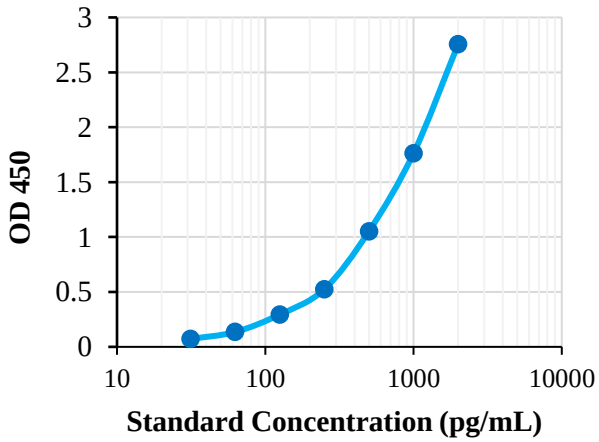
SPECIFICITY

This assay recognizes natural and recombinant human NT-proBNP.

RELATIVE PRODUCTS

Human CEA ELISA (TBS3210)
Human AFP ELISA (TBS3212)
Human HE4 ELISA (TBS3213)
Human IL-1ß ELISA (TBS3219)
Human IL-2 ELISA (TBS3220)
Human IL-4 ELISA (TBS3221)
Human IL-6 ELISA (TBS3223)
Human IL-7 ELISA (TBS3224)
Human IL-8 ELISA (TBS3225)
Human IL-13 ELISA (TBS3227)
Human IL-17 ELISA (TBS3228)
Human IL-22 ELISA (TBS3229)
Human IFN-gamma ELISA (TBS3230)
Human TGF- ß1 ELISA (TBS3232)
Human GM-CSF ELISA (TBS3233)
Human MIP-1α ELISA (TBS3234)
Human TNF-α ELISA (TBS3235)
Human IL-18 ELISA (TBS3239)

Fig3: Fast Human NT-proBNP ELISA



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