

Datasheet for ABIN368630  
**NT-ProBNP ELISA Kit**



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## Overview

Quantity:	96 tests
Target:	NT-ProBNP
Binding Specificity:	N-Term
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0.312-20 ng/mL
Minimum Detection Limit:	0.312 ng/mL
Application:	ELISA

## Product Details

Purpose:	For the quantitative determination of human N-terminal pro-brain natriuretic peptide (NT-proBNP) concentrations in serum, plasma, cell culture supernates, tissue homogenates.
Sample Type:	Serum, Plasma, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of human NT-proBNP.
Cross-Reactivity (Details):	Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between the target antigen and all analogues for other species. Therefore, cross reaction may still exist.
Sensitivity:	0.216 ng/mL

## Product Details

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Components:	<ul style="list-style-type: none"><li>• Assay plate (12 × 8 coated Microwells)</li><li>• Standard (freeze dried)</li><li>• Biotin-antibody (100 × concentrate)</li><li>• HRP-avidin (100 × concentrate)</li><li>• Biotin-antibody Diluent</li><li>• HRP-avidin Diluent</li><li>• Sample Diluent</li><li>• Wash Buffer (25 × concentrate)</li><li>• TMB Substrate</li><li>• Stop Solution</li><li>• Adhesive Strip (for 96 wells)</li><li>• Instruction manual</li></ul>
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## Target Details

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Target:	NT-ProBNP
Alternative Name:	Pro-Brain Natriuretic Peptide (NT-ProBNP) ( <a href="#">NT-ProBNP Products</a> )

## Application Details

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Application Notes:	<ul style="list-style-type: none"><li>• The supplier is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.</li><li>• 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.</li><li>• Grossly hemolyzed samples are not suitable for use in this assay.</li><li>• If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.</li><li>• Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.</li><li>• Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.</li><li>• Owing to the possibility of mismatching between antigens from another resource and antibodies used in this supplier's kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by this supplier's products.</li><li>• Influenced by factors including cell viability, cell number and cell sampling time, samples from cell culture supernatant may not be recognized by the kit.</li><li>• Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.</li></ul>
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## Application Details

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Comment: Detection wavelength: 450 nm

### Information on standard material:

Depending on the antigen to be detected, standards can be either native or recombinant protein. The recombinant proteins are being expressed in CHO cells in most cases. Please inquire for more information. The formulation of auxiliary material in the standard is considered proprietary information, however it does not contain any poisonous substance. Proclin 300 (1:3000) is used as preservative.

### Information on reagents:

In most cases the stop solution provided is 1 N H<sub>2</sub>SO<sub>4</sub>. The formulation of wash solution is proprietary information. None of the components contain (sodium) azide, thimerosal, 2-mercaptoethanol (2-ME) or any other poisonous materials. For the sandwich method kits, the sample diluent, antibody diluent, enzyme diluent and standard all contain BSA.

### Information on antibodies:

The antibodies provided in different kits vary in regards to clonality and host. Some antibodies are affinity purified, some are Protein A

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Sample Volume: 100 µL

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Assay Time: 1 - 4.5 h

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Plate: Pre-coated

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Protocol: This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for NT-proBNP has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any NT-proBNP present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for NT-proBNP is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of NT-proBNP bound in the initial step. The color development is stopped and the intensity of the color is measured.

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Reagent Preparation:

- **Biotin-antibody (1×)** - Centrifuge the vial before opening.  
Biotin-antibody requires a 100-fold dilution. The suggested dilution is 10µL of Biotin-antibody + 990µL of Biotin-antibody Diluent.
- **HRP-avidin (1×)** - Centrifuge the vial before opening.

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HRP-avidin requires a 100-fold dilution. The suggested dilution is 10µL of HRP-avidin + 990µL of HRP-avidin Diluent.

- **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 20mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500mL of Wash Buffer (1×).
- **Standard** - Centrifuge the standard vial at 6000-10000rpm for 30s.  
Reconstitute the Standard with 1ml of Sample Diluent. Do not substitute other diluents. This reconstitution produces a stock solution of 200pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.  
Pipette 250µL of Sample Diluent into each tube. Use the stock solution to produce a 2-fold dilution series. Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard (200pg/mL). Sample Diluent serves as the zero standard (0ng/mL).

Note:

- Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.
- Bring all reagents to room temperature (18-25°C) before use for 30 min.
- Prepare fresh standard for each assay. Use within 4 hours and discard after use.
- Making serial dilution in the wells directly is not permitted.
- Please carefully reconstitute Standards according to the instruction. Avoid foaming and mix gently until the crystals have completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µL when pipetting.
- It is recommended to use distilled water to prepare reagents and samples. Using contaminated water or container for reagent preparation will influence detection result.

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Assay Precision:	<p>Intra-assay precision (precision within an assay): Three samples of known concentration were tested twenty times on one plate to assess precision.</p> <p>Inter-assay precision (precision between assays): Three samples of known concentration were tested in twenty assays to assess precision.</p> <ul style="list-style-type: none"><li>• Intra-assay: CV% less than 8%</li><li>• Inter-assay: CV% less than 10%</li></ul>
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Restrictions:	For Research Use only
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Handling

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Precaution of Use:	The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face and clothing protection when using this material.
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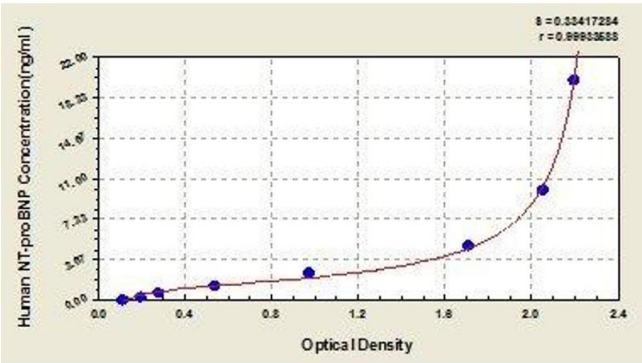
Handling

Handling Advice:	<ul style="list-style-type: none"><li>• The kit should not be used beyond the expiration date on the kit label.</li><li>• Do not mix or substitute reagents with those from other lots or sources.</li><li>• If samples generate values higher than the highest standard, dilute the samples with Sample Diluent and repeat the assay.</li><li>• Any variation in Sample Diluent, operator, pipetting technique, washing technique, incubation time/temperature and kit age can cause variation in binding.</li><li>• This assay is designed to eliminate interference by soluble receptors, binding proteins and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.</li></ul>
Storage:	4 °C/-20 °C
Storage Comment:	For unopened kit: All the reagents should be kept according to the labels on vials.
Expiry Date:	6 months

Publications

Product cited in:	Lee, Mudd, Gelow, Nguyen, Hiatt, Green, Denfeld, Bidwell, Grady: "Background and design of the profiling biobehavioral responses to mechanical support in advanced heart failure study." in: <b>The Journal of cardiovascular nursing</b> , Vol. 29, Issue 5, pp. 405-15, (2015) ( <a href="#">PubMed</a> ).
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Images



ELISA

Image 1. Typical standard curve



## Successfully validated (ELISA (ELISA))

by [Alamo Laboratories](#)

Report Number: 029606

Date: Feb 09 2014

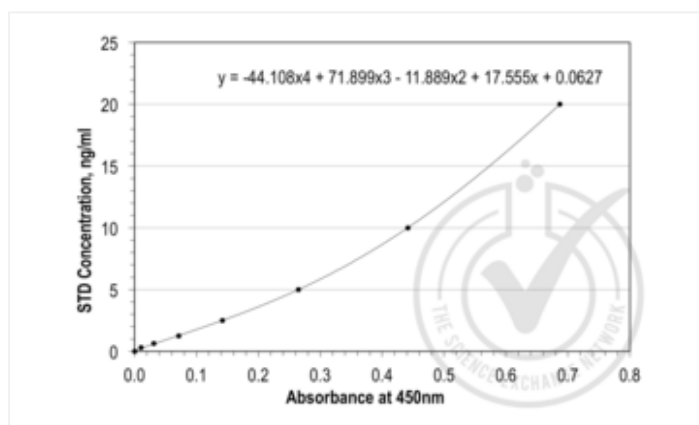
Lot Number:	U05095912
Method validated:	ELISA (ELISA)
Positive Control:	<a href="#">Human serum</a>
Negative Control:	Goat serum
Notes:	Signal was detected in positive control sample and not in negative control sample.
Primary Antibody:	- Antigen: Human Pro-Brain Natriuretic Peptide (NT-ProBNP) - Catalog number: ABIN368630 - Supplier: Cusabio - Supplier catalog number: CSB-E05152h - Lot number: U05095912
Controls:	<ul style="list-style-type: none"><li>• Positive control: Serum from normal adult human (specimen known to contain the target protein).</li><li>• Negative control: Serum from normal goat (specimens known to not contain the target protein).</li><li>• Standard curve: Serial two-fold dilutions from 20 ng/ml [20, 10, 5, 2.5, 1.25, 0.625, 0.312, 0] were generated from the standard provided in the kit using standard/sample diluent buffer.</li><li>• Spike control: Standard diluted in standard/PBS diluent buffer [2.5 and 0].</li></ul>
Protocol:	<ul style="list-style-type: none"><li>• All reagents in the ELISA kit were brought up to room temperature (RT) before use.</li><li>• 100 µL of standard or sample were added to wells in ELISA plate pre-coated with capture antibody. All samples and standards were assayed in triplicate.</li><li>• The plate was covered with sealer (provided in kit) and incubated for 2 hours at 37°C. Unbound material was aspirated but the wells were NOT Washed.</li><li>• 100 µL of Biotin-Antibody (diluted 1:100 in "Biotin-Antibody Diluent") was added to each well. Plate was covered with sealer (provided in kit) and incubated for 1 hour at 37°C. Unbound Biotin-Antibody was removed from each well and plate was washed three times with 350 µL of wash buffer (provided in the kit). After the last wash the plate was inverted against clean absorbent paper to remove any remaining liquid.</li><li>• 100 µL of HRP-Avidin Conjugate (diluted 1:100 in "HRP-Avidin Diluent") was added to each well. Plate was covered with sealer (provided in kit) and incubated for 1 hour at 37°C.</li><li>• Unbound HRP-Avidin was removed by washing five times with 350 µL of wash buffer (provided in the kit). After the last wash the plate was inverted and blotted against clean absorbent paper to remove any remaining liquid.</li><li>• 90 µL of TMB substrate was added to wells and the plate was covered with a new plate</li></ul>

sealer. The plate was gently tapped to ensure mixing and incubated for 30 min at 37°C in the dark.

- After 30 min, when an apparent gradient appeared in the standard wells, the reaction was terminated by adding 50 µL of Stop Solution to each well.
- The optical density (OD value) of each well was read using a microplate reader set to 450 nm.
- The triplicate readings for each sample were averaged and the average zero standard optical density subtracted to yield 'corrected absorbance at 450 nm'. A standard curve was generated by plotting the mean OD value for each standard on the X-axis against the concentration on the Y-axis using Excel. Standard curve was generated by regression analysis with four-parameter logistic.
- An equation ( $y = -44.108x^4 + 71.899x^3 - 11.889x^2 + 17.555x + 0.0627$ ) was derived from the standard curve and used to calculate NT-proBNP concentrations in samples based on their Average Absorbance values.

Experimental Notes: None

## Images for Validation report #029606



### Validation image no. 1 for Pro-Brain Natriuretic Peptide (NT-ProBNP) (NT-ProBNP) ELISA Kit (ABIN368630)

Figure 1: Graph of corrected OD 450 nm plotted for standard curve samples.

Type	Sample, ng/ml	Readings at 450 nm			Avg Reading	Corrected OD <sub>450nm</sub>	SD	Calculated conc ng/ml
		1	2	3				
Standards	20	0.781	0.698	0.792	0.757	0.687	0.042	20.00
	10	0.489	0.498	0.547	0.511	0.441	0.025	10.00
	5	0.321	0.338	0.345	0.335	0.265	0.010	4.99
	2.5	0.221	0.220	0.195	0.212	0.142	0.012	2.50
	1.25	0.130	0.148	0.146	0.141	0.071	0.008	1.28
	0.625	0.093	0.121	0.089	0.101	0.031	0.014	0.60
	0.312	0.080	0.080	0.082	0.081	0.011	0.001	0.25
	0	0.068	0.068	0.074	0.070	0.000	0.003	0.06
Spike Controls	2.50	0.197	0.220	0.189	0.202	0.132	0.013	2.32
	0.00	0.071	0.069	0.070	0.070	0.000	0.001	0.06
Test Samples	Serum, Human Male67yr	0.093	0.080	0.087	0.087	0.017	0.005	0.35
	Serum, Goat	0.070	0.068	0.065	0.068	-0.002	0.002	0.02

Conc of NT-proBNP in Serum, Human Male67yr (+ve Control) : 0.35 ng/ml  
Conc of NT-proBNP in Goat Serum (-ve Control) : 0.02 ng/ml

### Validation image no. 2 for Pro-Brain Natriuretic Peptide (NT-ProBNP) (NT-ProBNP) ELISA Kit (ABIN368630)

Table 1: ELISA. NT-proBNP is present in Human serum and undetectable in goat serum. Spike controls indicate no interference in absorbance readings from the diluent used to prepare standards and sera samples. Absorbance readings (OD 450 nm) are shown for standard curve, spike controls and unknown samples. Value for Avg Reading is derived from the average reading of three samples. Avg Reading for "0" amount of Standard was subtracted from all

Avg Readings to yield "Corrected OD450 nm" values for Standards, spike controls and unknown samples. Standard deviation is included for all samples. Standard curve was generated by regression analysis with four-parameter logistic. An equation ( $y = -44.108x^4 + 71.899x^3 - 11.889x^2 + 17.555x + 0.0627$ ) was derived from the standard curve and used to calculate NT-proBNP concentrations shown in Table 1.