Antibody and Immunoassay Services

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Human BNP Immunoassay Kit

Catalogue Number: 31660

For the quantitative determination of human BNP concentrations
in serum, plasma and cell culture supernatant samples.

This package insert must be read in its entirety before using this product.
Use only the current version of product data sheet enclosed with the kit.

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Version: 1.0

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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INTRODUCTION

Brain Natriuretic peptide (BNP) is a hormone secreted by cardiomyocytes in the ventricles in response to stretching caused by increased ventricular volume. Stimulation of the natriuretic peptide receptor by BNP triggers natriuresis, diuresis, vasodilation, inhibition of renin and aldosterone and inhibition of fibrosis. BNP is cleaved by kidney. Its half-life is around 20 minutes.

Many studies demonstrated that BNP is a biomarker of heart failure (HF). Elevating concentration of BNP is related to existence of HF. Normally, the circulating BNP concentration in the blood is around 30 – 50 pg/ml. BNP > 100 pg/ml has the diagnostic value for HF with the accuracy of 85 %. When the BNP exceeds 400 pg/ml, immediate medical treatment is required.

Beside HF, many other cardiopulmonary disorders are associated with elevated circulating BNP concentration: acute coronary syndrome, myocarditis, valvular heart disease, hypertrophic cardiomyopathy, cardiotoxic drugs, atrial fibrillation or flutter and right ventricular dysfunction in the setting of significant pulmonary disease.

PRINCIPLE OF THE ASSAY

This assay is a rapid quantitative sandwich ELISA. The immune-plate is pre-coated with a monoclonal antibody specific for human BNP. Standards or samples together with a biotin labelled monoclonal antibody specific for human BNP are pipetted into the wells and any human BNP present is bound by the immobilized antibodies. After washing away any unbound substances, streptavidin-HRP conjugate (STP-HRP) is added. After the last wash step, an HRP substrate solution is added and colour develops in proportion to the amount of human BNP bound initially. The assay is stopped and the optical density of the wells determined using a microplate reader. Since the increases in absorbance are directly proportional to the amount of captured human BNP, the unknown sample concentration can be interpolated from a reference curve included in each assay.

INTENDED USE

This human BNP ELISA kit is designed for quantification of human BNP in serum, plasma and cell culture supernatant samples.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. Micro-titre Strips (96 wells)-Coated with a monoclonal antibody against human BNP, sealed.
2. 10×Wash buffer-50 ml.
3. 5×Assay buffer-20 ml.
4. 100×Detection antibody solution-A biotin labelled monoclonal antibody against human BNP, 0.12 ml.
5. Human BNP standard-1000 pg of recombinant human BNP in a buffered protein base, lyophilised.
6. 200×STP-HRP solution- 0.06 ml.
7. Substrate solution- 12 ml, ready for use.
8. Stop solution- 12 ml, ready for use.
9. Plate Cover- 1

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips.
2. 96-well plate or manual strip washer.
3. Buffer and reagent reservoirs.
4. Paper towels or absorbent paper.
5. Plate reader capable of reading absorbency at 450 nm.
6. Distilled water or deionized water.
7. Horizontal micro-plate shaker capable of 600 rpm

STORAGE

The kit should be stored at 2-8°C upon receipt, and all reagents should be equilibrated to room temperature before use. Remove any unused antibody-coated strips from the human BNP microplate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 1×Assay buffer.

Prepare 1×Assay buffer by mixing the 5×Assay buffer (20 ml) with 80 ml of distilled water or deionized water. If precipitates are observed in the 5× Assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Assay buffer may be stored at 2-8°C for up to one month.

B. 1×Wash buffer.

Prepare 1×Wash buffer by mixing the 10×Wash buffer (50 ml) with 450 ml of distilled water or deionized water. If precipitates are observed in the 10× Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Wash buffer may be stored at 2-8°C for up to one month.

C. 1×Detection antibody solution.

Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 μl of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is removed.

D. 1×STP-HRP solution.

Spin down the 200×STP-HRP solution briefly and dilute the desired amount of the 200×STP-HRP solution 1:200 with 1×Assay buffer, 100 μl of the 1×STP-HRP solution is required per well. Prepare only as much 1×STP-HRP solution as needed. Return the 200×STP-HRP solution to 2-8°C immediately after the necessary volume is removed.

PREPARATION OF STANDRADS AND SAMPLES

Human BNP standards: Reconstitute the lyophilised standard with 1 ml of 1×Assay buffer to generate a standard stock solution of 1000 pg/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare serially diluted standards using 1×Assay buffer as follows:

Standard volume	Volume of 1×Assay buffer	Concentration
1000 pg/ml stock	-	1000 pg/ml
250 µl of 1000 pg/ml	250 µl	500 pg/ml
250 µl of 500 pg/ml	250 µl	250 pg/ml
250 µl of 250 pg/ml	250 µl	125 pg/ml
250 µl of 125 pg/ml	250 µl	62.5 pg/ml
250 µl of 62.5 pg/ml	250 µl	31.25 pg/ml
250 µl of 31.25 pg/ml	250 µl	15.625 pg/ml
250 µl of 15.625 pg/ml	250 µl	7.8125 pg/ml

1×Assay buffer serves as the zero standard (0 pg/ml).

Note: The reconstituted standard stock should be aliquoted and stored at -80ºC for up to one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample preparation

Serum or plasma sample is generally required a 4-fold dilution in the 1×Assay buffer. BNP basal expression is low and may not be able to be detected in normal samples of human serum or plasma.

Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

ASSAY PROCEDURE

It is recommended that all standards and samples should be assayed in duplicate.

1. Add 50 µl of standard or sample and 50 µl of 1×Detection antibody solution per well, incubate at room temperature for 1 hour with, shaking the plate at 600 rpm on a horizontal micro-plate shaker
2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 320 µl of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 4 washes.
3. Add 100 µl of 1×STP-HRP solution to each well, incubate at room temperature for 20 minutes.
4. Wash each well 5 times as described in step 2.
5. Add 100 µl of Substrate solution to each well, incubate at room temperature for 10 minutes. **Protect from light.**
6. Add 100 µl of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
7. Measure absorbance of each well at 450 nm immediately.

CALCULATION

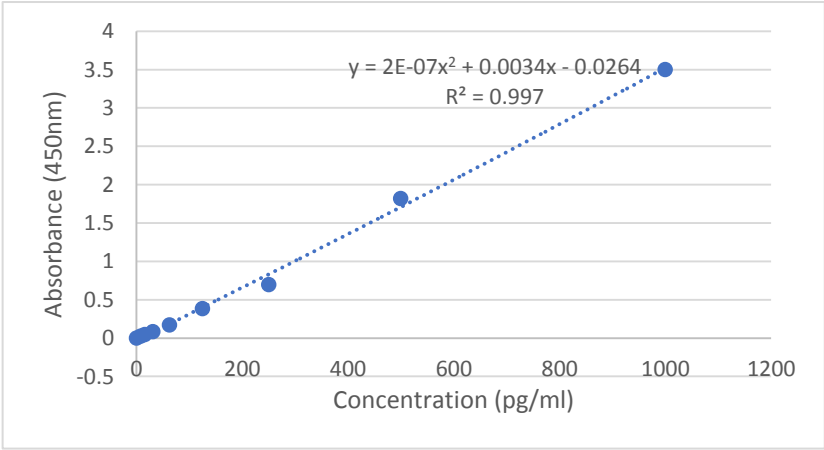
1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against human BNP concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine human BNP concentration of samples from standard curve and multiply the value by the dilution factor.

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each set of sample assay. *

Human BNP (pg/ml)	Absorbance (450 nm)	Blanked Absorbance
0	0.125	0
7.8125	0.140	0.025
15.625	0.172	0.047
31.25	0.210	0.085
62.5	0.295	0.170
125	0.509	0.384
250	0.821	0.696
500	1.944	1.819
1000	3.627	3.502

Human BNP standard curve (2-parameter)



*If only 7 points are required for the STD curve, the customer can omit one of the concentration expect the highest and lowest value when creating your own STD curve.

ASSAY CHARACTERISTICS

A. Sensitivity:

Limit of Detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank plus two standard deviations of the absorbance of blank: $AVG._{blank} + 2*SD$) is calculated from the real BNP values in wells and is 4.285 pg/ml.

B. Precision:

Intra-assay Precision (Precision within an assay)

Two samples of known concentration were tested 20 times on one plate.

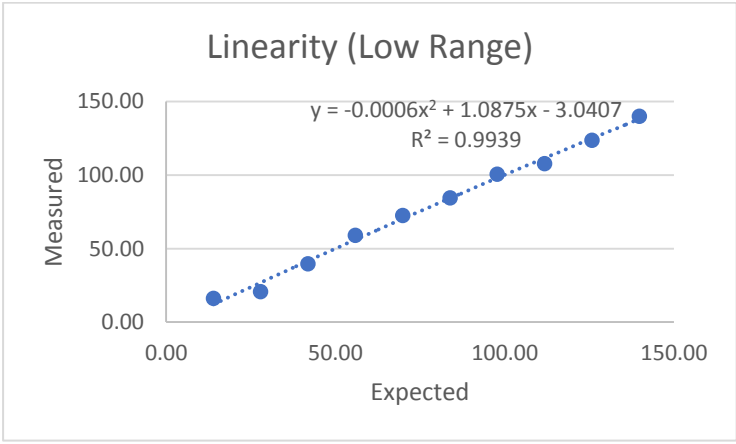
Sample	n	Mean (pg/ml)	SD (pg/ml)	CV (%)
1	20	93.8	5.09	5.4
2	20	53.7	4.51	8.4

C. Linearity:

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human BNP were serially diluted with the 1×Assay buffer to produce samples with values within the dynamic range of the assay.

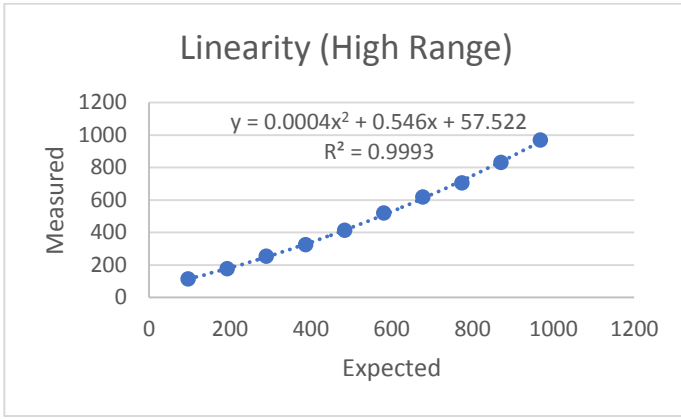
Low Range

Dilution	Measured	Expected	Recovery
1	139.82	139.82	100%
0.9	123.57	125.84	98%
0.8	107.62	111.86	96%
0.7	100.42	97.88	103%
0.6	84.46	83.89	101%
0.5	72.43	69.91	104%
0.4	59.00	55.93	105%
0.3	39.58	41.95	94%
0.2	20.72	27.96	74%
0.1	16.10	13.98	115%



High Range

Dilution	Measured	Expected	Recovery
1	967.94	967.94	100%
0.9	830.60	871.15	95%
0.8	704.24	774.35	91%
0.7	617.65	677.56	91%
0.6	520.07	580.76	90%
0.5	412.93	483.97	85%
0.4	323.66	387.18	84%
0.3	254.32	290.38	88%
0.2	176.96	193.59	91%
0.1	113.17	96.79	117%



Linearity of Human BNP ELISA range from 13.98 to 967.94 pg/ml, R^2>0.99

D. Spiking:

3 different concentrations of human BNP STD are spiked into the 4-fold diluted serum samples to check for the recovery rate.

				Serum	Assay Buffer	
	Serum	Conc.	Add	Test result	Expected	recovery
1st trail	0	0	0	0	0	
	0	250	13.34	11.73	13.34	88%
	0	500	43.97	34.59	43.97	79%
	0	1000	83.74	80.65	83.74	96%
2nd trial	0	0	0	0	0	
	0	250	15.46	13.76	15.46	89%
	0	500	45.98	36.65	45.25	81%
	0	1000	81.25	76.24	81.98	93%

The recovery rate ranges from 79% to 96%

SUMMARY OF ASSAY PROCEDURE

Add 50 µl of Standard or sample to each well.

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Add 50 µl of 1×Detection antibody solution to each well.

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Incubate at room temperature for 1 hour.

↓

Aspirate and wash each well four times.

↓

Add 100 µl of 1×STP-HRP solution to each well.

↓

Incubate at room temperature for 20 minutes.

↓

Aspirate and wash each well five times.

↓

Add 100 µl of Substrate solution to each well.

↓

Incubate at room temperature for 10 minutes.

↓

Add 100 µl of Stop solution to each well.

↓

Measure absorbance of each well at 450 nm.

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Calculation