***For Research Use Only Not for Diagnostic Use***

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**Human BNP ELISA Kit**

**Catalog No. EA200023**

**Principle of the Assay**

Brain natriuretic peptide (BNP) is a hormone secreted by cardiomyocytes in the heart ventricles in response to stretching caused by increased ventricular blood volume. BNP is synthesized as a 134-amino acid preprohormone (preproBNP), encoded by the human gene NPPB. Removal of the 25-residue N-terminal signal peptide generates the prohormone, proBNP, which is subsequently cleaved into a biologically inactive 76–amino acid N-terminal fragment (NT-proBNP) and the biologically active 32-amino acid polypeptide BNP. Once released, BNP binds to and activates the atrial natriuretic factor receptor NPRA. BNP was found to have an essential role in the prognostication of heart surgery patients and may be a reliable predictor of cardiovascular mortality in diabetics. BNP has been toas an aid in the diagnosis and assessment of severity of heart failure.

This sandwich ELISA is used to measure human BNP in serum, plas,ma and other biological fluids. Microtitration wells coated with anti-human BNP capture antibody are exposed to test specimens. The BNP antigen in the specimen is specifically captured onto the immobilized antibody during specimen incubation. The captured BNP antigen is then reacted with HRP conjugated human BNP detection antibody. After washing, specifically bound enzyme conjugate is detected by reaction with the Substrate Solution, tetramethylbenzidine (TMB). The assay is measured spectrophotometrically to indicate the level of BNP present in a sample.

# Materials Supplied

# The reagents supplied in this pack are for Research Use Only.

|  |  |
| --- | --- |
| **Description** | Quantity |
| BNP Antibody Coated 96-well Plate in foil pouch with desiccant | 1 |
| Recombinant Human BNP Standard (100ng/mL) | 100 µL |
| HRP Conjugated BNP Detection Antibody (100x) | 120 µL |
| Assay Buffer | 15 mL |
| BNP Standard Diluent | 20 mL |
| Sample Diluent 1 | 20 mL |
| Sample Diluent 2 | 20 mL |
| Substrate Solution (TMB) | 12 mL |
| Stop Solution (1N HCl) | 12 mL |
| Wash Buffer (20x) | 60 mL |
| Plate Sealer | 2 |

**Additional Materials not Supplied**

1. Horizontal orbital plate shaker capable of maintaining a speed of 450±50 rpm.
2. Disposable pipet tips to deliver volumes of 5µL, 10 µL, 25 µL, 100 µL and 200 µL (multichannel pipette preferred for dispensing reagents into microtiter plates).
3. Distilled or deionized water.
4. Clean, disposable plastic/glass test tubes, approximate capacities 5mL and 10mL.
5. Range of standard, clean volumetric laboratory glassware consisting of, at least 15 mL and 100 mL beakers, 1 L graduated cylinder, 1 mL, 5 mL, and 10 mL pipettes.
6. Absorbent paper towels.
7. Automatic microplate washer or laboratory wash bottle.
8. Microplate reader with 450nm filter.
9. Latex gloves, safety glasses, and other appropriate protective garments.
10. Biohazard waste containers.
11. Safety pipetting devices for 1 mL or larger pipettes.
12. Timer.

**Storage and Stability**

Upon receipt, store the kit at 2-8°C. The kit should not be used beyond the expiration date. Once opened, the unused microplate strips should be returned to their original foil pouch along with the desiccant. The diluted Wash Buffer should not be stored for longer than 3 weeks at 2-8°C. It is recommended that Wash buffer be freshly diluted before each assay. If the diluted Wash buffer becomes visibly cloudy during the 3 weeks, discard it. (Note: Concentrated Wash Buffer, when stored at 2-8°C, normally may develop crystalline precipitates, which can be re-dissolved at 37°C.)

*Indications of Deterioration*

The human BNP Assay kit may be considered to have deteriorated if:

1. Reagents are visibly cloudy.

2. The Substrate Solution turns blue. This is likely to be caused by chemical contamination of the Substrate Solution.

**Precautions**

1. The reagents supplied in this kit are for Research use only.

2. All blood products should be treated as potentially infectious.

2. Disposal or decontamination of fluid in the waste reservoir should be in accordance with guidelines described in the Department of Labor, Occupational Safety and Health Administration, occupational exposure to blood-borne pathogens; final rule (29 CFR 1910,1030) FEDERAL REGISTER, pp. 64176-84177,12/6/91.

3. The Substrate Solution and Stop Solution in this kit can irritate the skin and cause eye damage. Handle them with care and wear protective gloves, clothing and eye/face protection. Wash hands thoroughly after handling. Immediately flush the affected area with plenty of water in case of contact with skin or eyes. Obtain medical attention if necessary.

**Technical Suggestions**

1. This kit should be used in strict accordance with the instructions in the Package Insert.
2. Do not use the kit after the expiration date printed on the outer carton label.
3. Do not cross contaminate reagents.
4. Some reagents in the BNP ELISA kit are optimized for each kit lot. Do not exchange reagents from kits with different lot numbers.
5. To ensure accurate results and avoid cross-contamination, use proper adhesive plate sealers during incubation steps, and change pipette tips when adding each standard and sample. Multi-channel pipettes are recommended for large assays. Always use fresh pipette tips when drawing from stock reagent bottles.
6. Warm up the foil bag to room temperature before opening.
7. All reagents should be added to the plate in the same order.
8. If the Stop Solution does not mix thoroughly with the Substrate Solution, the color in the wells may appear green after adding stop solution. Gently tap the plate or pipette up and down to mix until the color in the wells change to yellow (avoid bubbles during this step).
9. Reagents should be dispensed with the tip of the micropipettes touching the side of the well at a point about mid-section. For automatic processors, follow manufacturer's recommendations.
10. It is recommended that all pipetting devices (manual or automatic), and thermometers are regularly calibrated according to the manufacturer's instructions.

**Sample Collection and Storage**

The Human BNP ELISA is intended for use with serum, plasma and other biological fluids. The specimen should be tested as soon as possible. However, if the specimen needs storage, the specimens should be stored frozen at -20°C or below. Do not use self-defrosting freezers. Specimens that have been frozen and thawed should be thoroughly mixed before testing.

**Serum**: Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Plasma**: Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Cell culture supernatants and other biological fluids**: Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Rinse Cycle**

Aspirate each well and wash. Wash by filling each well with Wash Buffer (300μL) using a squirt bottle, manifold dispenser, or automatic plate washer. Complete removal of liquid at each step is essential to good performance. After the last wash, invert the plate and blot it against clean paper towels.

**Preparation for the Assay**

1. *Standard preparation*: Prepare protein standard by diluting 10µL of standard stock into 490 µL (1:50 dilution) of Standard Diluent. This will give a final concentration of 2000pg/mL as shown in Table 1. Make 2x serial dilution of Standard 1 using Standard Diluent to generate a standard concentration range of 31.25 to 2000 pg/mL.

**Table 1: Human BNP Standard Curve Generation**

|  |  |  |  |
| --- | --- | --- | --- |
| **Standard Number** | **Concentration of BNP (pg/mL)** | **BNP Standard (µL)** | **Standard Diluent (µL)** |
| 1 | 2000 | 10 | 490 |
| 2 | 1000 | 250 of #1 | 250 |
| 3 | 500 | 250 of #2 | 250 |
| 4 | 250 | 250 of #3 | 250 |
| 5 | 125 | 250 of #4 | 250 |
| 6 | 62.5 | 250 of #5 | 250 |
| 7 | 31.25 | 250 of #6 | 250 |
| 8 | 0 |  | 250 |

2. *Sample preparation*: BNP concentration must be estimated prior to performing the full experiment by testing a serially diluted representative sample using Sample Diluent 1 (for serum and EDTA-plasma), Sample Diluent 2 (for Heparin-plasma), or Standard Diluent (for culture supernatant). Select an optimal dilution level such that the final target protein concentration falls near the middle of the assay linear dynamic range. Or refer to published literature for expected concentrations and derive the optimal dilution level based on the expected dynamic range of the kit. A 2-fold dilution is recommended for normal serum and plasma sample.

3. *HRP Conjugated BNP Detection Antibody preparation*: dilute the concentrated HRP antibody conjugate 1:100 using assay buffer.

4. *Wash buffer* *preparation*: Prepare working-strength Wash buffer by diluting 1 part concentrate with 19 parts of distilled or de-ionized water. If a kit is likely to be utilized over a period in excess of 4 weeks, then it is recommended that only enough stock concentrate be diluted sufficient for immediate needs.

**Assay Procedure**

**Note: All standards, controls and samples should be tested in duplicate.**

1. Allow all reagents to reach room temperature (18-25°C).

2. Select sufficient microtitration well strips to accommodate all test specimens, controls and reagent blank. Fit the strips into the holding frame.

3. Dispense 100 µL of each standard and sample into appropriate wells. Note: All standards and samples should be tested in duplicate. Note: Depending on the BNP concentration of your sample, dilute your sample using corresponding sample diluent.

4. Incubate for 2 hours at room temperature with moderate shaking (450±50rpm) on a horizontal orbital plate shaker.

5. Wash the microtitration plate 4 times as described in the Rinse Cycle section.

6. Pipette 100 µL of 1x HRP conjugated detection antibody into each well and incubate for 1 hour at room temperature with moderate shaking (450±50rpm) on a horizontal orbital plate shaker.

7. Wash the microtitration plate 4 times as described in the Rinse Cycle section.

8. Dispense 100 µL Substrate Solution into each well. A multichannel pipette should be used for best results. Leave at room temperature (18-25°C) and protected from direct sunlight for 20-25 minutes.

9. Stop the reaction by adding 100 µL of Stop Solution to each well. The blue solution should change to a uniform yellow color. Ensure that the undersides of the wells are dry and that there are no air bubbles in the well contents.

10. Immediately after adding the Stop solution, read the absorbance values at 450 nm using a microtitration plate reader.

**Calculation of Results**

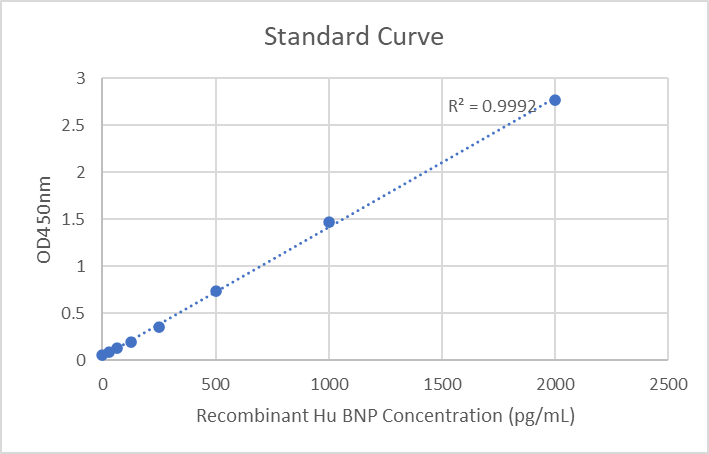
Average the duplicate optical density (O.D.) readings for each standard and sample. A 4-parameter logistic (4-PL) or a linear regression model providing a point-to-point curve fitting provides acceptable results. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) or a linear regression 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. Do not force the line to be linear. The concentration of the specimens can be found directly from the standard curve.

**Table 2. Example Data at 450nm.**

|  |  |
| --- | --- |
| **Standards** | **450 nm absorbance** |
| Standard 1 (2000 pg/mL) | 2.771 |
| Standard 2 (1000 pg/mL) | 1.47395 |
| Standard 3 (500 pg/mL) | 0.7396 |
| Standard 4 (250 pg/mL) | 0.3563 |
| Standard 5 (125 pg/mL) | 0.1997 |
| Standard 6 (62.5 pg/mL) | 0.12925 |
| Standard 7 (31.25 pg/mL) | 0.0927 |
| Standard 8 (0 pg/mL) | 0.06285 |

**Typical Human BNP ELISA Kit Standard Curve**

This standard curve was generated at OriGene for demonstration purpose only. A standard curve must be run with each assay.



**Add TMB Substrate**

**Incubate 30 minutes**

**Wash 6 times**

**Add SA-HRP**

**Incubate 30 minutes**

Note: This standard curve is only an example and should not be used to generate any results.

**Performance Characteristics**

1. **Recovery**

The recovery of human BNP spiked to three different-levels of the assay range in diluted samples was evaluated.



1. **Linearity**

To assess the linearity of the assay, spiked human samples were diluted to produce samples with values within the dynamic range of the assay.



1. **Sensitivity:** 6.99pg/mL
2. **Precision**

Samples with different levels of BNP were assayed 10 times each on three different assays. The intra-assay CV percentage and inter-assay CV percentage were calculated.

Intra-assay



Inter-assay

**Limitations of Use**

1. This kit is **not for use in diagnostic procedures**.

2. Assay values determined using assays from different manufacturers or different methods may not be used interchangeably.

3. The assay cannot be used to quantitate samples with BNP assay values greater than the highest standard without further serial dilution of the samples.

# Contact Information:

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**Assay Flowchart**

**Add 100 µL of Stds and Samples**

**Incubate 2 hours at RT with Shaking**

Wash 4 times towetowelstowelsttttttoweltowels

**Add 100 µL of 1x HRP conjugated Detection Antibody**

**Incubate 1 hour at RT with Shaking**

Wash 4 times towels

**Add 100 µL of TMB Substrate**

**Incubate 20-25 minutes at RT**

**Add 100 µL of Stop Solution**

**Read in Plate Reader**