ARBOR ASSAYS™
Interactive Assay Solutions™

DetectX®

Human B-Type Natriuretic Peptide (BNP)
ELISA Kit

1 Plate Kit Catalog Number K083-H1
5 Plate Kit Catalog Number K083-H5

Sample Types Validated:

Serum and Plasma

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

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BACKGROUND

B-type natriuretic peptide (BNP [formerly known as brain natriuretic peptide]) is a member of the natriuretic peptide family, which also includes atrial-NP (ANP) and C-type NP (CNP).[1,3] BNP was originally discovered in porcine brain and later identified in the heart and blood circulation.[1] In human, the precursor of BNP, known as pre-pro BNP protein, is produced in heart ventricle cardiomyocytes in response to pressure/volume overload and cytokine signals.[3] Secondary cleaving of pre-pro BNP produces pro-BNP (BNP 1-108).[3] Pro-BNP is then cleaved into biologically active BNP, a 32 amino acid polypeptide, and the 76 amino acid N-terminal pro-BNP (NT-proBNP), both of which are released into circulation.[3]

Biologically active BNP levels are tightly regulated by precise hormonal control.[4] Circulating BNP, along with ANP, binds and activates the transmembrane guanylyl cyclase natriuretic peptide receptor-A (NPRA), producing cyclic GMP.[3] Cyclic GMP then stimulates cGMP-dependent protein kinase (PKG), inducing vasodilation and other biological responses. [3-5]

Both BNP and NT-proBNP are applied as diagnostic, managing, and prognostic tools for heart failure (HF).[6-10] BNP levels greater than 100 pg/mL can be indicative of HF while above 430 pg/mL is a predictor for mortality.[6,10] Studies have observed increased BNP plasma levels in patients with cancer, providing further consideration when using BNP as a heart failure biomarker for these patients.[11]

1. Aburaya, M., et al. (1985). “Distribution and molecular forms of brain natriuretic peptide in porcine heart and blood”, *Biochem Biophys Res Commun*, 165 (2), 872-9.
2. Sudoh, T., et al. (1988). “A new natriuretic peptide in porcine brain”, *Nature*, 332 (6159): 78-81.
3. Potter, L., et al. (2009). “Natriuretic peptides: Their structures, receptor, physiologic functions and therapeutic applications, *Handb Exp Pharmacol*, (191), 341-366.
4. George, J. & Struthers, A.D., (2007). “Natriuretic peptides”, *Comprehensive Hypertension*, 349-362.
5. Pandey, K. N. (2005). “Biology of natriuretic peptides and their receptors”, *Peptides*, 26 (6), 901-932.
6. Palazzuoli, A., et al. (2010). “Natriuretic peptides (BNP and NT-proBNP): measurement and relevance in heart failure”, *Vasc Health Risk Manag*, (6), 411-8.
7. Authors/Task Force Members, et al. (2008). “ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM), *European Heart Journal*, Vol 29 (19), 2388-2442.
8. Maisel, A. S., et al. (2002). “Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure”, *N Engl J Med*, 347(3), 161-167.
9. Buchan, T. A., et al. (2021). “Prognostic value of natriuretic peptides in heart failure: systematic review and meta-analysis”, Heart Fail Rev, 27(2), 645-654.
10. Fonarow, G. C., et al. (2007). “Admission B-Type Natriuretic Peptide Levels and in-hospital mortality in acute decompensated heart failure”, *Journal of the American College of Cardiology*, Vol 49 (19), 1943-1950.
11. Bando, S., et al. (2017). “Plasma brain natriuretic peptide levels are elevated in patients with cancer”, *Plos one*, 12(6), e0178607.

ASSAY PRINCIPLE

The DetectX® Human B-Type Natriuretic Peptide (BNP) ELISA Kit is designed to quantitatively measure BNP present in serum and plasma samples. Please read the complete kit insert before performing this assay. A BNP standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve.

Standards or diluted samples are added to a clear microtiter plate coated with a monoclonal antibody to capture BNP present in the sample. A peroxidase-conjugated BNP monoclonal antibody is added and the plate is incubated for 2 hours. The plate is washed and substrate added, which reacts with the bound BNP-conjugated antibody. After 30 minutes, the substrate reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450 nm wavelength. The concentration of BNP in the sample is calculated, after making suitable correction for any dilution, using software available with most plate readers.

RELATED PRODUCTS

DetectX® Kits	Catalog No.
Atrial Natriuretic Peptide (ANP) ELISA Kits	K071-H1/H5
C-Reactive Protein (CRP) Human ELISA Kits	K069-H1/H5
Cyclic GMP Direct ELISA Kits	K020-H1/H5
Cyclic GMP Chemiluminescent ELISA Kits	K020-C1/C5
Cyclic GMP Direct ELISA Kits - Improved Sensitivity	K065-H1/H5
Endothelin-1 (ET-1) ELISA Kits	K045-H1
Hemoglobin High Sensitivity Colorimetric Detection Kits	K013-HX1/HX5
Myeloperoxidase (MPO) Human ELISA Kit	K060-H1
Prostaglandin E_2 (PGE_2) Multi-Format ELISA Kits	K051-H1/H5

SUPPLIED COMPONENTS

Sheep Anti-BNP Clear Coated 96 Well Plate

Clear plastic microplate with break-apart strips coated with sheep anti-human B-Type Natriuretic Peptide.

K083-H1 or -H5	1 or 5 Each	Catalog Number C309-1EA

Human BNP Standard

A stock solution of synthetic human B-Type Natriuretic (1-32) Peptide at 8,000 pg/mL in a special stabilizing solution.

K083-H1 or -H5	40 µL or 200 µL	Catalog Number C313-40UL or -200UL

DetectX® BNP Conjugate

A sheep anti-human monoclonal antibody to B-Type Natriuretic Peptide labeled with peroxidase.

K083-H1 or -H5	3 mL or 13 mL	Catalog Number C308-3ML or -13ML

Assay Buffer Concentrate

A 5X concentrate that should be diluted with deionized or distilled water.

K083-H1 or -H5	28 mL or 55 mL	Catalog Number X067-28ML or -55ML

Wash Buffer Concentrate

A 20X concentrate that should be diluted with deionized or distilled water.

K083-H1 or -H5	30 mL or 125 mL	Catalog Number X007-30ML or -125ML

TMB Substrate

K083-H1 or -H5	11 mL or 55 mL	Catalog Number X019-11ML or -55ML

Stop Solution

A 1M hydrochloric acid solution. **CAUSTIC**.

K083-H1 or -H5	5 mL or 25 mL	Catalog Number X020-5ML or -25ML

Plate Sealer

K083-H1 or -H5	1 or 5 Each	Catalog Number X002-1EA

STORAGE INSTRUCTIONS

The unopened kit must be stored at -20°C. Once opened the kit can be stored at 4°C up to the expiration date on the kit label**, except for the Human BNP Standard. This must be stored at -20°C.**

OTHER MATERIALS REQUIRED

Distilled or deionized water.

Polypropylene test tubes or vials for preparing standards and sample dilutions. **Do not use glass.**

Repeater pipet with disposable tips capable of dispensing 25 µL and 100 µL.

A microplate washer.

A microplate shaker capable of shaking around 700-900 rpm.

Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.

Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

Protease Inhibitor as indicated in Sample Preparation on page 7 for long-term storage of samples.

PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The antibody coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.

This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure **all** buffers used for samples are **azide free**. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on page 8.

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

SAMPLE TYPES

This assay has been validated for human serum and plasma samples only. Samples containing visible particulate should be centrifuged prior to using. The end user should test this kit for application in their samples.

Please refer to Cross Reactivity on page 14 to learn more about applicability to other species.

SAMPLE PREPARATION

Serum and Plasma Samples

Serum and plasma samples must be diluted ≥ 1:10 in diluted Assay Buffer before running in the assay. Adjusted for a minimum dilution of serum and plasma, the assay will measure from 800-12.5 pg/mL BNP. If elevated levels are expected, additional dilution in Assay Buffer may be necessary.

Collection

Collect with a pre-chilled blood collection tube (serum or EDTA plasma).

Centrifuge at refrigerated temperature and immediately aliquot into plastic tubes. Keep serum and plasma chilled or on ice until used or prepare for longer-term storage within 7 hours from time of collection.

Storage of Samples

Samples can be stored at room temperature for up to 7 hours if stored on ice. Store serum and plasma ≤ -20°C for longer term storage after addition of Protease Inhibitors.

The following Protease Inhibitors MUST be added to all samples prior to long-term storage:

- Phenylmethanesulfonyl fluoride (PMSF), such as Sigma 78830 at 100 mM in ethanol, diluted to 1 mM in samples.
- A universal protease inhibitor cocktail (PIC), such as Sigma P1860 or Roche 05892970001, diluted 1:200 in samples, or according to manufacturer's instructions.

Use all samples within 1 hour of dilution.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. Keep standard stock solution chilled on ice or at -20°C when not in use. Prepare all BNP dilutions in polypropylene tubes or vials. **Do not use glass.**

Assay Buffer

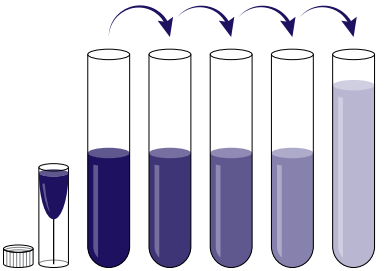
Dilute Assay Buffer Concentrate 1:5 by adding 1 part of the concentrate to 4 parts of deionized water. Once diluted, this is stable for 3 months at 4°C.

Wash Buffer

Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to 19 parts of deionized water. Once diluted, this is stable at room temperature for 3 months.

Standard Preparation

Label polypropylene tubes as #1 through #7. Pipet 990 µL of diluted Assay Buffer into tube #1. Pipet 200 µL of diluted Assay Buffer into tubes #2 to #7. **The BNP Standard stock solution contains an organic solvent. Pre-rinse the pipet tip several times to ensure accurate delivery.** Carefully add 10 µL of Standard Stock to tube #1 and vortex completely. Take 200 µL of the solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #7. The concentration of BNP in tubes #1 through #7 will be 80, 40, 20, 10, 5, 2.5, and 1.25 pg/mL.



	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer Volume (µL)	990	200	200	200	200	200	200
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Volume of Addition (µL)	10	200	200	200	200	200	200
Final Conc (pg/mL)	80	40	20	10	5	2.5	1.25

Use all Standards within 2 hour of preparation. Discard diluted standards.

Prepare all reagents, standards and samples prior to starting the assay. BNP binding to the antibody on the plate starts as soon as samples or standards are added to the well.

ASSAY PROTOCOL

Ensure that all chilled samples have reached room temperature and have been diluted in polypropylene tubes prior to running them in the kit.

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine BNP concentrations.

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification.
2. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.
3. **Assay binding reaction starts as soon as the first sample or standard is added.** Have pipet tools ready and load standards or samples and conjugate efficiently. Pipet 50 µL of samples or standards into wells in the plate. Pipet 50 µL of diluted Assay Buffer into the zero standard wells.
4. Add 25 µL of the DetectX® BNP Conjugate to each well using a repeater pipet.
5. Cover the plate with the plate sealer and incubate at room temperature for **2 hours with shaking.** We recommend shaking at around 700-900 rpm. Non-shaking will reduce ODs by approximately 15%**.**
6. Aspirate the plate and wash each well 4 times with 300 µL of diluted Wash Buffer. Tap the plate dry on clean, absorbent towels.
7. Add 100 µL of the TMB Substrate to each well, using a repeater pipet.
8. Incubate the plate at room temperature for 30 minutes without shaking.
9. Add 50 µL of the Stop Solution to each well, using a repeater pipet.
10. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
11. Use the plate reader's built-in 4PLC software capabilities to calculate the BNP concentration for each sample.

NOTE: If you are using only part of a strip well plate, at the end of the assay throw away the used wells and retain the plate frame for use with the remaining unused wells.

CALCULATION OF RESULTS

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic curve (4PLC) fit. The sample concentrations should be multiplied by the dilution factor to obtain neat sample valves.

Or, use the online tool from MyAssays to calculate the data:
https://www.myassays.com/arbor-assays-detectx-human-b-type-natriuretic-peptide-(bnp)-elisa.assay

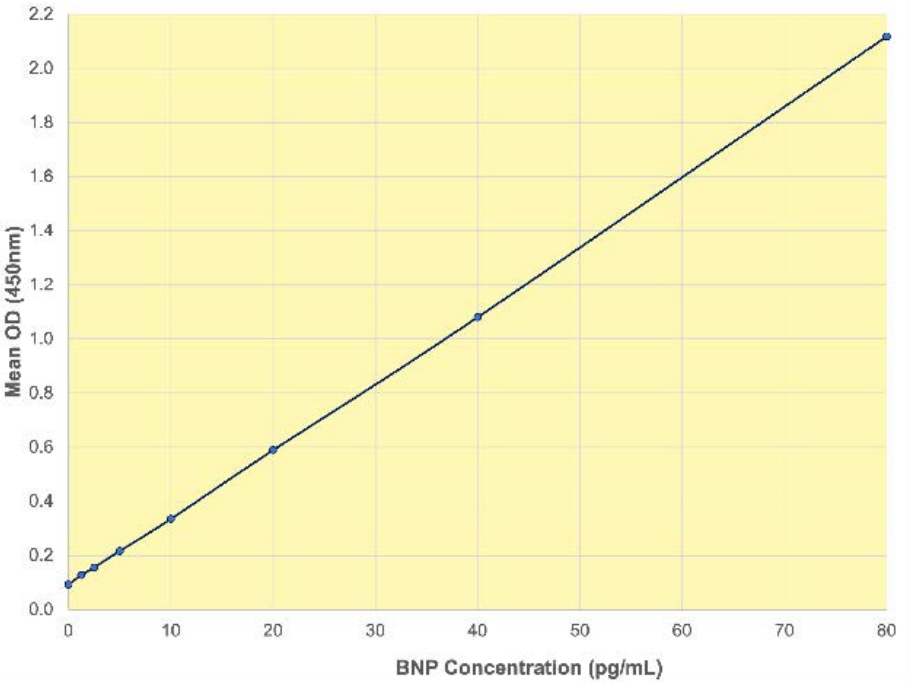
TYPICAL DATA

Sample	Mean OD	BNP Conc. (pg/mL)
Standard 1	2.119	80
Standard 2	1.081	40
Standard 3	0.590	20
Standard 4	0.336	10
Standard 5	0.217	5
Standard 6	0.157	2.5
Standard 7	0.128	1.25
Zero	0.094	0
Sample 1	0.515	17.2
Sample 2	0.919	33.3

Always run your own standard curve for calculation of results. Do not use this data.

Conversion of 1 pg/mL equals 0.406 pM (pmol/L).

Typical Standard Curve



Always run your own standard curves for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity and Limit of Detection

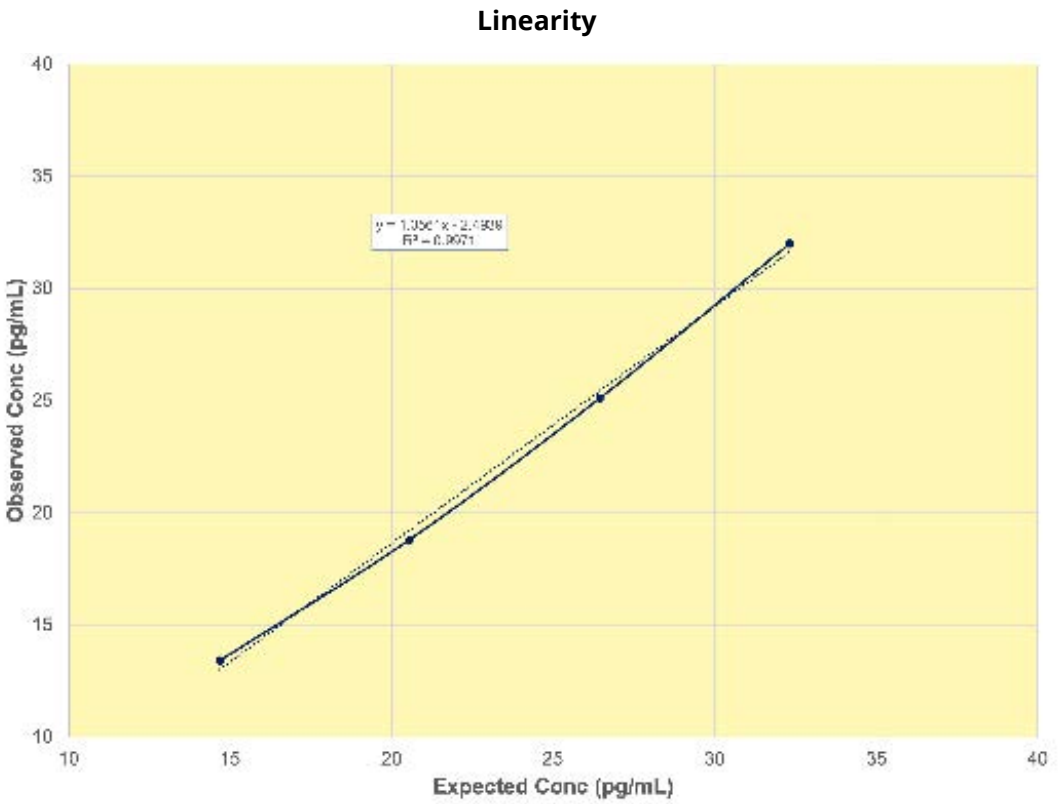
Sensitivity was calculated by comparing the OD's for 19 wells run for each of the zero and standard #7. The detection limit was determined at two (2) standard deviations from the zero standard along the standard curve. Sensitivity was determined as 0.31 pg/mL.

The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for 20 runs for each of the zero standard and a low concentration human sample. Limit of Detection was determined as 0.26 pg/mL.

Linearity

Linearity was determined by taking two serum samples, one with a low BNP level of 8.8 pg/mL and one with a higher level of 38.2 pg/mL and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High Serum	Low Serum	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80%	20%	32.3	32.0	99.0
60%	40%	26.4	25.1	94.9
40%	60%	20.6	18.8	91.4
20%	80%	14.7	13.4	91.3
			Mean Recovery	**94.2%**



Intra Assay Precision

Three samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated BNP concentrations were:

Sample	BNP Conc. (pg/mL)	%CV
1	6.9	3.2
2	16.4	2.9
3	33.6	3.4

Inter Assay Precision

Three samples were diluted with Assay Buffer and run in duplicates in twenty assays run over multiple days by several operators. The mean and precision of the calculated BNP concentrations were:

Sample	BNP Conc. (pg/mL)	%CV
1	7.0	13.9
2	17.0	9.3
3	32.6	8.1

High Dose Hook

Samples were prepared with BNP concentrations as high as 500,000 pg/mL and tested in the assay. Bound optical density levels indicated that high BNP concentrations would not give artificially depressed BNP concentrations.

SAMPLE VALUES

A number of serum and plasma samples from presumed heart failure patients were tested in the assay. Adjusted neat BNP concentrations ranged from 19 to almost 2,000 pg/mL, with an average of 498 pg/mL.

Reference ranges for serum/plasma in humans are ≤ 35-93 pg/mL for adult males and ≤ 64-167 pg/mL for adult females per Mayo Clinic Laboratories.[1] Ranges greater than normal up to 200 pg/mL are likely compensated congestive heart failure (CHF), ≤ 400 pg/mL are likely moderate CHF, and levels > 400 pg/mL are likely moderate to severe CHF.

1. Mayo Clinic Laboratories. Test ID: BNP. B-Type Natriuretic Peptide, Plasma. (https://www.mayocliniclabs.com/test-catalog/overview/83873#Clinical-and-interpretive).
2. Bio-Rad Laboratories, Inc., http://www.myeinserts.com/87840, Revision Date 2022-04-05.

CROSS REACTIVITY

BNP is the 32 amino acid fragment of the pre-pro-BNP protein. Researchers should perform and rely on cross reactivity with validation experiments to determine if the kit has the ability to read BNP in species other than humans.

The following cross reactants were tested in the assay and calculated on the standard curve.

Steroid	Cross Reactivity (%)
Human BNP	100.00%
Pro BNP	16.67%
Mouse BNP	<0.001%
ANP	<0.001%
CNP	<0.001%
NT-Pro BNP	<0.001%
Urodilatin	<0.001%

Five serum and plasma samples from people classified as having cardiac heart failure were diluted and tested in the kit. BNP levels ranged from 51.4 to 975.3 pg/mL. QC controls were made by diluting Bio-Rad LiquiCheck ™ Cardiac Markers Plus Control Levels 1,2 and 3 and found to read within the reference ranges of 79.9-158 pg/mL, 307-748 pg/mL and 1,207-3,254 pg/mL respectively.

LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

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OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with EIA kits for wildlife conservation research.

ASSAYS™
FOR SERIOUS SCIENCE

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