

ARBOR ASSAYS™  
Interactive Assay Solutions™



**DetectX<sup>®</sup>**

## **Atrial Natriuretic Peptide (ANP) Enzyme Immunoassay Kit**

1 Plate Kit Catalog Number K071-H1

5 Plate Kit Catalog Number K071-H5

**Species Independent**

**New Antibody**

**Sample Types Validated:**

**Plasma, Urine and  
Tissue Culture Media**

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

[www.ArborAssays.com](http://www.ArborAssays.com)   

**K071-H 191211**

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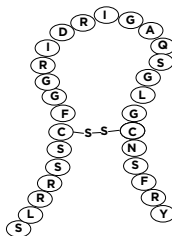
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## BACKGROUND

Atrial Natriuretic Peptide (ANP) was discovered in the early 1980s and described in publications in 1983/4<sup>1,2</sup>. ANP is derived from a 151 amino acid long protein called preproANP<sup>3</sup>. The prepro protein is cleaved to generate proANP. The principle storage form of the peptide is the proANP form which is 126 amino acids long. ANP is derived from amino acids 99-126 to form the 28 amino acid peptide with a disulfide bond between amino acids 7 and 23. ANP is the predominant member of a family of structurally and functionally related peptide hormones that exert a wide array of effects on cardiovascular and renal function. The combined actions of ANP on vasculature, kidneys, and adrenals serve both acutely and chronically to reduce systemic blood pressure as well as intravascular volume<sup>4,5</sup>. ANP and the related brain natriuretic peptide bind to their common receptor, membrane-type guanylyl cyclase-A, which leads to biological actions through a cGMP-dependent pathway.

**Atrial Natriuretic Peptide**



1. de Bold AJ, and Flynn TG. "Cardionatrin I-a novel heart peptide with potent diuretic and natriuretic properties.", 1983, Life. Sci., 33:297-302.
2. Kangawa K, and Matsuo H. "Purification and complete amino acid sequence of alpha-human atrial natriuretic polypeptide (alphaANP)." 1984, Biochem. Biophys. Res. Commun., 118:131—139.
3. Oikawa, S, et al., "Cloning and sequence analysis of cDNA encoding a precursor of human atria1 natriuretic polypeptide.", 1984, Nature, 309: 724-726.
4. Ballerman, BJ., and Brenner. BM, "Biologically active atrial peptides.", 1985. J. Clin. Invest., 76:2041-2048.
5. Ballerman, BJ., and Brenner. BM. "Role of atria1 peptides in body fluid homeostasis.", 1986, Circ. Res., 58: 619-630

## ASSAY PRINCIPLE

*Please read the complete kit insert before performing this assay.*

The DetectX<sup>®</sup> ANP Immunoassay Kit is designed to quantitatively measure ANP present in plasma, urine, and tissue culture media samples. An ANP 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 pipetted into a clear microtiter plate coated with an antibody to capture rabbit antibodies. An ANP-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of an antibody to ANP to each well. After an hour incubation the plate is washed and substrate is added. The substrate reacts with the bound ANP-peroxidase conjugate. After a short incubation, the 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 the ANP in the sample is calculated, after making suitable correction for the dilution of the sample, using software available with most plate readers.

## RELATED PRODUCTS

Kits	Catalog No.
C-Reactive Protein (CRP) Human ELISA Kits	K069-H1/H5
Creatinine Serum Detection Kits	KB02-H1/H2
Cyclic GMP Direct ELISA Kits	K020-H1/H5
Cyclic GMP Direct Chemiluminescent Kits	K020-C1/C5
Cyclic GMP Direct ELISA Kits (Improved Sensitivity)	K065-H1/H5
Hemoglobin High Sensitivity Detection Kits	K013-HX1/HX5
Myeloperoxidase (MPO) Human ELISA Kit	K060-H1
Prostaglandin E <sub>2</sub> (PGE <sub>2</sub> ) ELISA Kits	K051-H1/H5
Urea Nitrogen (BUN) Detection Kits	K024-H1/H5



## SUPPLIED COMPONENTS

### Coated Clear 96 Well Plates

Clear plastic microtiter plate(s) coated with goat anti-rabbit IgG.  
Kit K071-H1 or -H5 1 or 5 Each

Catalog Number X016-1EA

### ANP Standard

ANP at 1,800 ng/mL in a special stabilizing solution.  
Kit K071-H1 or -H5 125 µL or 625 µL

Catalog Number C095-125UL or -625UL

### DetectX® ANP Antibody

A rabbit polyclonal antibody specific for ANP.  
Kit K071-H1 or -H5 3 mL or 13 mL

Catalog Number C262-3ML or -13ML

### DetectX® ANP Conjugate

An ANP-peroxidase conjugate in a special stabilizing solution.  
Kit K071-H1 or -H5 3 mL or 13 mL

Catalog Number C094-3ML or -13ML

### Assay Buffer Concentrate

Assay Buffer, 5X concentrate that should be diluted with deionized or distilled water.  
Kit K071-H1 or -H5 28 mL or 55 mL

Catalog Number X065-28ML or -55ML

### Extraction Solution

A special solution for treatment of plasma samples to extract ANP.  
Kit K071-H1 or -H5 50 mL or 250 mL

Catalog Number X123-50ML or -250ML

### Wash Buffer Concentrate

A 20X concentrate that should be diluted with deionized or distilled water.  
Kit K071-H1 or -H5 30 mL or 125 mL

Catalog Number X007-30ML or -125ML

### TMB Substrate

Kit K071-H1 or -H5 11 mL or 55 mL

Catalog Number X019-11ML or -55ML

### Stop Solution

A 1M solution of hydrochloric acid. **CAUSTIC.**  
Kit K071-H1 or -H5 5 mL or 25 mL

Catalog Number X020-5ML or -25ML

### Plate Sealer

Kit K071-H1 or -H5 1 or 5 Each

Catalog Number X002-1EA

## STORAGE INSTRUCTIONS

All components of this kit should be stored at 4°C until the expiration date of the kit.

## OTHER MATERIALS REQUIRED

Distilled or deionized water.

Glass or high-quality polypropylene tubes.

A Speedvac/centrifugal concentrator or N<sub>2</sub> gas and gas manifold for evaporation.

Protease inhibitor cocktail (PIC), such as Sigma P1860-1ML (to add to plasma samples).

Repeater-type pipet with disposable tips capable of dispensing 25, 50, and 100 µL.

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.

## 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 plasma, urine, and for tissue culture media samples. Samples containing visible particulate should be centrifuged prior to using.

ANP is identical in a large number of species. Human, pig, sheep and dog ANP have identical sequences and this kit will measure samples from these species identically. Rat and mouse ANP molecules differ at amino acid 12 where isoleucine replaces methionine in human ANP and show 100% cross reactivity in the kit. The end user should evaluate recoveries of ANP in other sample matrices being tested.

## SAMPLE PREPARATION

### Plasma Samples

Plasma samples should be extracted with the provided Extraction Solution, or with a solid phase C18 column extraction protocol (see Peptide/Protein Extraction Protocol at [www.ArborAssays.com/resources/#protocols](http://www.ArborAssays.com/resources/#protocols)), prior to running in the kit.

#### Protocol Using Extraction Solution

1. Mix 1 part sample with 1.5 parts of Extraction Solution.
2. Vortex and then nutate at room temperature for 90 minutes.
3. Centrifuge for 20 minutes at 4°C at 1660 x g.
4. Transfer supernatant to a clean tube.
5. Speedvac supernatant to dryness at 37°C.
6. Reconstitute sample with 250 µL of diluted Assay Buffer.

### Urine Samples

Urine samples should be diluted  $\geq 1:5$  with the diluted Assay Buffer before running in the kit. For normalization to creatinine as a urine volume marker, please see our NIST-calibrated 2-plate and 10-plate Urinary Creatinine Detection kits, K002-H1 and K002-H5.

### Tissue Culture Media

For measuring ANP in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM depending on ANP levels. We have validated the assay using RPMI-1640.

**Use all diluted samples within 2 hours of preparation.**

## REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

### Assay Buffer

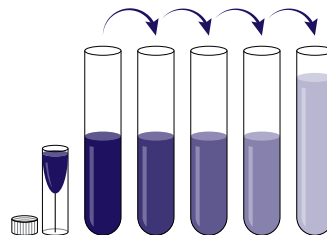
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

### Wash Buffer

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

### Standard Preparation

Label test tubes as #1 through #6. Pipet 450  $\mu\text{L}$  of Assay Buffer into tube #1 and 200  $\mu\text{L}$  into tubes #2 to #6. **The ANP stock solution contains an organic solvent. Pre-rinse the pipet tip several times to ensure accurate delivery.** Carefully add 50  $\mu\text{L}$  of the ANP stock solution to tube #1 and vortex completely. Take 100  $\mu\text{L}$  of the ANP solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #6. The concentration of ANP in tubes 1 through 6 will be 180, 60, 20, 6.67, 2.22, and 0.741 ng/mL.



**Use all Standards within 2 hour of preparation.**

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
<b>Assay Buffer Volume (<math>\mu\text{L}</math>)</b>	<b>450</b>	200	200	200	200	200
<b>Addition</b>	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
<b>Volume of Addition (<math>\mu\text{L}</math>)</b>	<b>50</b>	100	100	100	100	100
<b>Final Conc (ng/mL)</b>	180	60	20	6.67	2.22	0.741

## ASSAY PROTOCOL

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

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to foil pouch with desiccant. Seal the ziploc plate bag and store at 4°C.
2. Pipet 50 µL of samples or standards into wells in the plate.
3. Pipet 75 µL of Assay Buffer into the non-specific binding (NSB) wells.
4. Pipet 50 µL of Assay Buffer into the maximum binding (B0 or Zero standard) wells.
5. Add 25 µL of the DetectX® ANP Conjugate to each well using a repeater pipet.
6. Add 25 µL of the DetectX® ANP Antibody to each well, **except the NSB wells**, using a repeater pipet.
7. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 1 hour.
8. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
9. Add 100 µL of the TMB Substrate to each well using a repeater pipet.
10. Incubate the plate at room temperature for 30 minutes without shaking.
11. Add 50 µL of the Stop Solution to each well using a repeater pipet.
12. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
13. Use the plate reader's built-in 4PLC software capabilities to calculate ANP 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 the 4PLC fitting routine on the plate reader, after subtracting the mean OD's for the NSB. The sample concentrations obtained, calculated from the %B/B0 curve, should be multiplied by the dilution factor to obtain neat sample values.

Or use the online tool from MyAssays to calculate the data:

### TYPICAL DATA

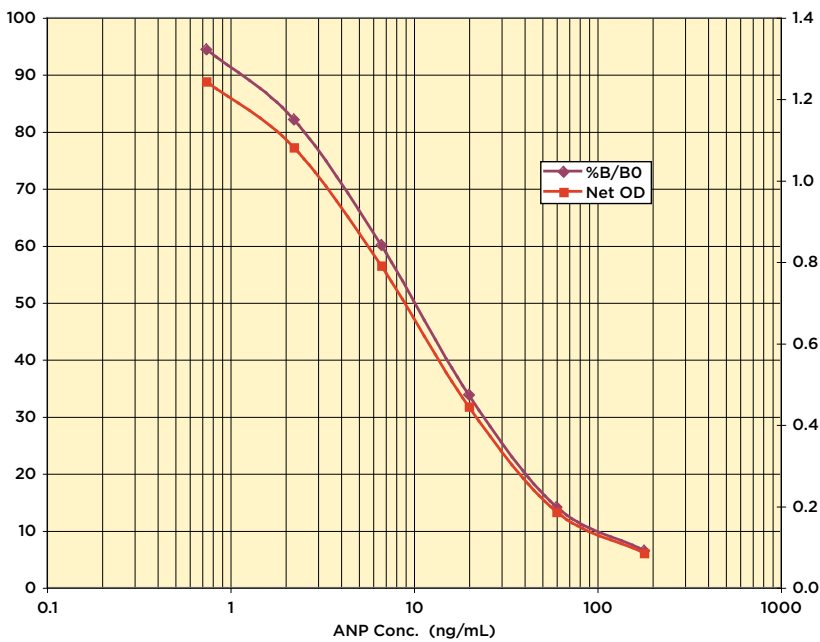
Sample	Mean OD	Net OD	% B/B0	ANP Conc. (ng/mL)
NSB	0.090	0.000	-	-
Standard 1	0.175	0.085	6.5	180
Standard 2	0.276	0.186	14.1	60
Standard 3	0.535	0.445	33.8	20
Standard 4	0.881	0.791	60.1	6.67
Standard 5	1.171	1.081	82.1	2.22
Standard 6	1.333	1.243	94.5	0.741
B0	1.406	1.316	100.0	0
Sample 1	0.456	0.366	27.8	26.2
Sample 2	0.981	0.891	67.7	4.8

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

**Conversion Factor: 100 ng/mL of human ANP is equivalent to 32.5 nM.**



## Typical Standard Curves



**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 twenty wells run for each of the B0 and standard #6. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve.

**Sensitivity was determined as 0.49 ng/mL.**

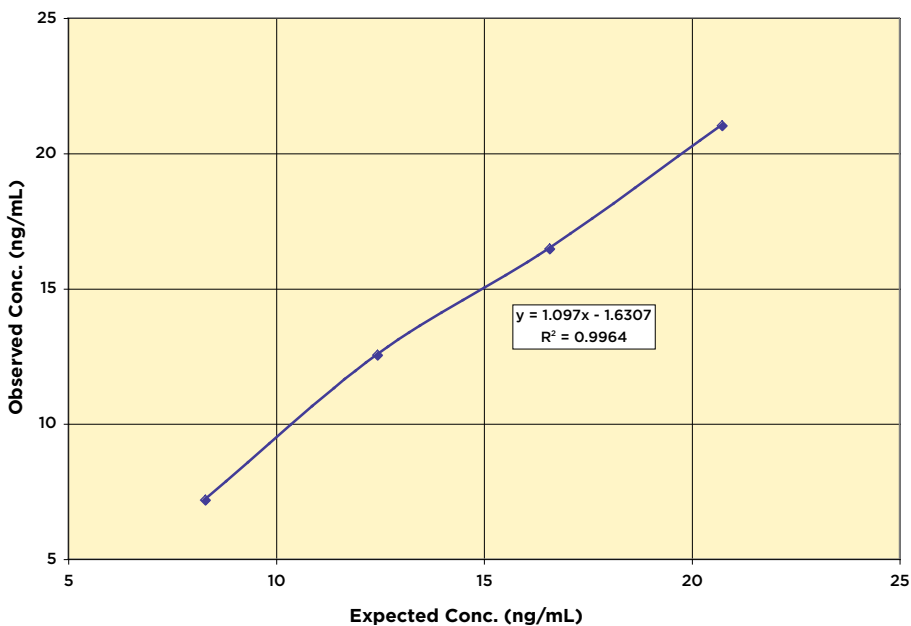
The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration human sample. **Limit of Detection was determined as 0.34 ng/mL.**

## Linearity

Linearity was determined by taking two urine samples diluted with Assay Buffer, one with a low diluted ANP level of 6.2 ng/mL and one with a higher diluted level of 29.3 ng/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

Low Urine	High Urine	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
20%	80%	24.7	25.6	103.6
40%	60%	20.0	20.8	104.0
60%	40%	15.4	16.4	106.5
80%	20%	10.8	11.8	109.3
<b>Mean Recovery</b>				<b>105.8%</b>

## Linearity



### Intra Assay Precision

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

Sample	ANP Conc. (ng/mL)	%CV
1	27.2	3.3
2	11.3	4.8
3	5.33	8.3

### Inter Assay Precision

Three human samples were diluted with Assay Buffer and run in duplicates in simh assays run over multiple days by five operators. The mean and precision of the calculated ANP concentrations were:

Sample	ANP Conc. (ng/mL)	%CV
1	26.4	8.7
2	10.9	9.2
3	4.9	8.3

## SAMPLE VALUES

Normal urine samples were diluted and run in the assay. Values ranged from 5.9 to 10.3 ng/mL with an average of 8.2 ng/mL.

Normal plasma samples were extracted and run. Values ranged from 0.84 to 3.0 ng/mL with an average for the samples of 1.4 ng/mL.

## INTERFERENTS

A variety of detergents and solvents were tested as possible interfering substances in the assay.

Addition Tween 20	% Added 0.10%	% Change in Measured ANP 6.2%
Chaps	0.01%	-6.6%
CTAC	0.004%	-2.5%
SDS	0.004%	-3.0%
Triton-X100	0.01%	-5.9%
Acetonitrile	1%	5.1%
DMF	0.5%	9.8%
DMSO	0.5%	9.2%
Ethanol	1%	-0.4%
Methanol	1%	0.9%

## CROSS REACTIVITY

The following cross reactants were tested in the assay and calculated at the 50% binding point.

Cross Reactant	Cross Reactivity (%)
Human ANP (1-28)	100%
Rat ANP (1-28)	99.4%
Rat ANF (8-33)	100%
Urodilantin	161.4%
Human $\beta$ -ANP (1-28)	50%
Human $\gamma$ -ANP	40%
Rat ANF (18-28)	60%
Atriopeptin II	5%
BNP	< 0.001%

*Note: The antibodies used in ANP ELISA, catalog numbers K026-H1/H5 and K071-H1/H5, show similar sensitivity. However, slight differences in cross-reactivity were observed.*



## 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

For details concerning this kit or to order any of our products please contact us:

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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 assay kits and reagents for wildlife conservation research.

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