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Atrial Natriuretic Peptide (ANP) ELISA Kit

rev 11/20

(Catalog # E4349-100, 100 assays, Store at 4°C)

I. Introduction:

Atrial Natriuretic Peptide (ANP) is derived from a 151 amino acid long protein called preproANP3. The preproprotein is cleaved to generate pro-ANP. This principle storage form of the peptide is the pro-ANP 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. ANP and the related brain natriuretic peptide bind to their common receptor, membrane-type guanylyl cyclase-A and binding leads to biological actions through a cGMP-dependent pathway. BioVision's ANP ELISA kit is a competitive ELISA assay for the quantitative measurement of ANP in extracted serum and plasma, or in urine, extracted dried fecal samples, and tissue culture media samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of ANP
Detection Range: 180 - 0.741 ng/ml
Sensitivity: < 0.25 ng/ml
Detection Limit: 0.26 ng/ml

III. Specificity:

Universal

IV. Sample Type:

Plasma, Urine and Tissue Culture Media

V. Kit Contents:

Components	E4349-100	Part No.
Micro ELISA Plate	8 X 12 strips	E4349-100-1
Standard	125 µl	E4349-100-2
ANP Antibody	3 ml	E4349-100-3
ANP Conjugate	3 ml	E4349-100-4
Assay Buffer Concentrate (5X)	28 ml	E4349-100-5
Extraction Solution	50 ml	E4349-100-6
Wash Buffer Concentrate (20X)	30 ml	E4349-100-7
TMB Substrate	11 ml	E4349-100-8
Stop Solution	5 ml	E4349-100-9
Plate Sealer	1	E4349-100-10

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Ethyl acetate or ethanol for serum, plasma or fecal extracts
- Speedvac for evaporation of ethanol or ethyl acetates
- Precision pipettes with disposable tips

VII. Storage and Handling:

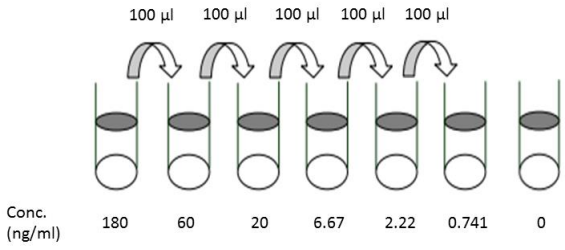
The entire kit may be stored at 4°C for up to 6 months. Avoid freeze-thaw cycles.

VIII. Reagent Preparation:

Note: Prepare reagents within 30 minutes before the experiment.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **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.
2. **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 for 3 months at room temperature.
3. **Standard Preparation:**

- Add 50 µl of the ANP stock solution to 450 µl of Assay Buffer (tube #1) and vortex completely.
- Prepare 5 vials of standards (tube #2-6) by adding 100 µl of the above stock solution in 200 µl of Assay Buffer. Perform serial dilutions of the top standards to make the standard curve within the range of this assay.



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- Suggested standard points are: 180, 60, 20, 6.67, 2.22, 0.741, 0 ng/ml.
  - Use all Standards within 2 hours of preparation.

4. Sample Preparation:

Note: Use all Samples within 2 Hours of preparation, or stored at ≤ -20°C until assaying. Avoid multiple freeze-thaw cycles.

- **Extracted serum and plasma**: Mix 1 part sample with 1.5 parts of Extraction Solution. Vortex and then incubate at room temperature for 90 minutes. Centrifuge for 20 minutes at 4°C at 1660 x g. Transfer supernatant to a clean tube. Speedvac supernatant to dryness at 37°C. Reconstitute sample with 250 µl of Assay Buffer.
- **Urine**: Urine samples should be diluted at least 1:5 with the provided Assay Buffer before running in the kit.
- **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.

IX. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VIII.
2. Pipet 50 µl of samples or standards into wells in the plate. Pipet 75 µl of Assay Buffer into the non-specific binding (NSB) wells.
3. Add 25 µl of the ANP Conjugate to each well. Add 25 µl of the ANP Antibody to each well, except the NSB wells.
4. 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.
5. Aspirate the plate and wash each well 4 times with 300 µl wash buffer. Tap the plate dry on clean absorbent towels.
6. Add 100 µl of the TMB Substrate to each well. Incubate the plate at room temperature for 30 minutes without shaking.
7. Add 50 µl of the Stop Solution to each well.
8. Read the optical density at 450 nm within 15 minutes.

X. CALCULATION:

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 non-specific binding well (NSB). The sample concentrations obtained, calculated from the $\%B/B_0$ curve, and should be multiplied by the dilution factor to obtain neat sample values.

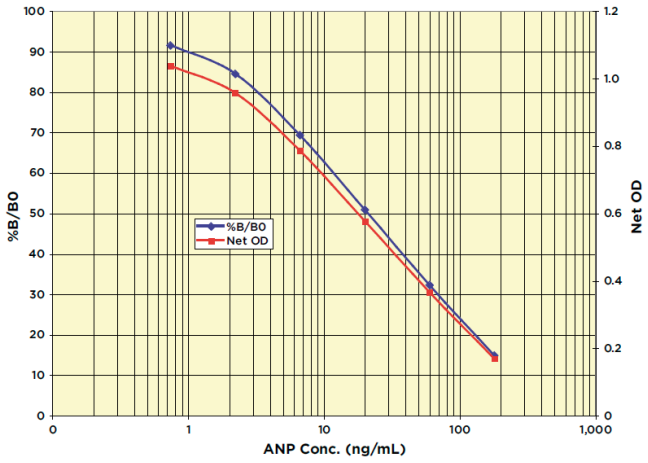

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

XI. VALIDATION DATA:

Recovery Rate:

Low Urine	High Urine	Observed Conc. (ng/mL)	Expected Conc. (ng/mL)	% Recovery
80%	20%	7.2	8.3	86.4
60%	40%	12.5	12.4	100.7
40%	60%	16.5	16.6	99.2
20%	80%	21.0	20.7	101.4
			Mean Recovery	**96.9%**

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Intra Assay:

Sample	ANP Conc. (ng/mL)	%CV
1	26.4	9.0
2	10.3	4.9
3	4.17	6.1

Inter Assay Precision:

Sample	ANP Conc. (ng/mL)	%CV
1	26.1	4.8
2	10.3	7.8
3	4.36	10.2

Interferents:

Addition	% Added	% Change in Measured ANP
Triton-X100	0.10%	9.2%
Chaps	0.01%	-9.1%
SDS	0.05%	-10.0%
CTAC	0.0004%	-8.6%
Ethanol	1%	-1.4%
Methanol	1%	-9.3%
DMSO	1%	3.4%
DMF	1%	5.3%
Acetonitrile	1%	9.2%

Cross Reactivity:

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

XII. RELATED PRODUCTS:

- cGMP Direct Immunoassay Kit (Colorimetric) (Cat. No. K372)
- cGMP Antibody (Cat. No. 3568)
- Creatinine Colorimetric/Fluorometric Assay Kit (Cat. No. K625)
- Albumin-to-Creatinine Ratio (ACR) Assay Kit (Cat. No. K551)

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