Atrial Natriuretic Peptide (ANP) Competitive ELISA Kit

Catalog Number EIAANP (96 tests)

Pub. No. MAN0024886 **Rev** A.0

For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Atrial Natriuretic Peptide (ANP) ELISA Kit is a solid-phase competitive Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of ANP in plasma, urine and tissue culture media. The assay recognizes ANP independent of species.

Atrial Natriuretic Peptide (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.

Contents and storage

Kit and components shipped at –20°C. Upon receipt, store the kit at –20°C. Once open, store kit at 4°C and use within 2 weeks.

Components	Quantity
ANP Standard; 1,800 ng/mL ANP in a special stabilizing solution	125 µL
Assay Buffer Concentrate (5X)	28 mL
Antibody Coated Wells, 96-well strip-well plate coated with goat anti-rabbit IgG	1 plate
ANP Antibody	3 mL
ANP Conjugate	3 mL
Extraction Solution	50 mL
Wash Buffer Concentrate (20X)	30 mL
TMB (Tetramethylbenzidine) Substrate	11 mL
Stop Solution; contains 1 M HCl, **CAUSTIC**	5 mL
Plate Sealer	1

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm (preferably with correction between 570 nm and 590 nm)
- Plate Shaker
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.
- Solutions containing sodium azide will inhibit the activity of the peroxidase conjugate. Ensure that there is no contamination of labware or the plate washer with azide containing solutions.

Prepare 1X Wash Buffer

1. Dilute 15 mL of Wash Solution Concentrate (20X) with 285 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 3 months.

Prepare 1X Assay Buffer

1. Dilute 14 mL of Assay Buffer (5X) with 56 mL of deionized or distilled water. Label as 1X Assay Buffer.
2. Store the concentrate and 1X Assay Buffer in the refrigerator. 1X Assay Buffer is stable at 4°C for 3 months.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera.
- If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Prepare samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

Use all samples within **2 hours** of dilution, or store at –20°C or lower until ready to perform assay.

Sample type	Procedure
Plasma	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 1,660 × *g*. 4. Speedvac supernatant to dryness at 37°C. 5. Reconstitute sample with 250 µL of Assay Buffer.
Urine	Dilute samples ≥1:5 with 1X Assay Buffer. **Note**: A Urinary Creatinine Detection Kit (Cat. no. EIACUN) is available for measuring urine creatinine for normalization of ANP in a random urine specimens.
Tissue culture media	Perform sample dilutions with the corresponding tissue culture medium.

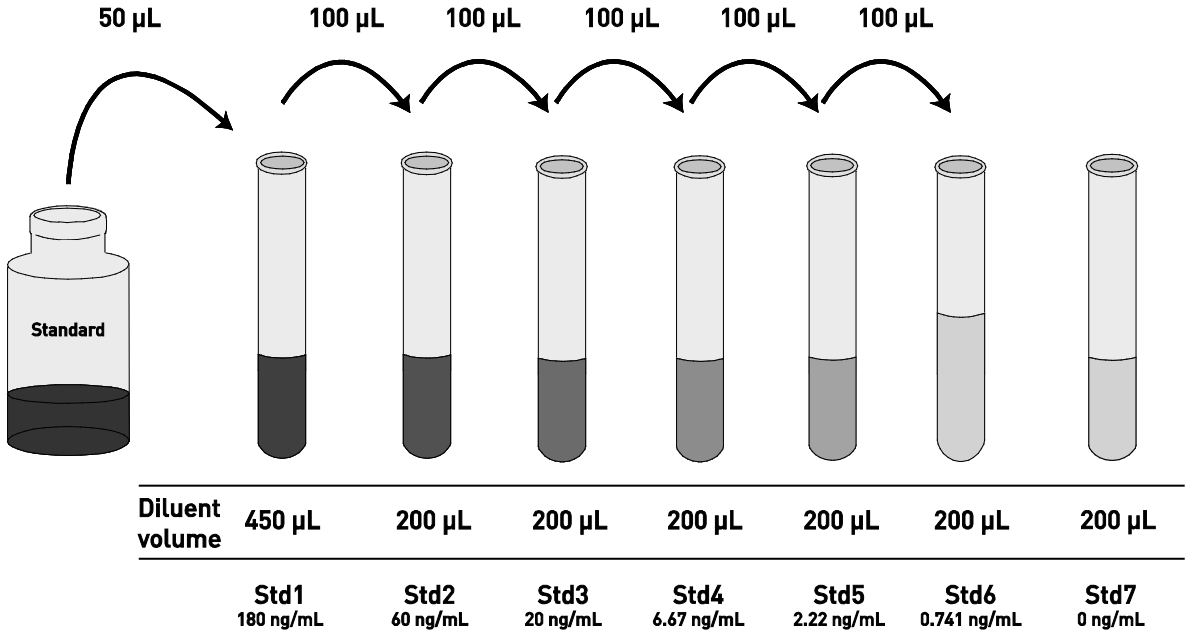
Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Instructions are for diluting standards from 180 to 0.741 ng/mL, but a curve can be obtained using a range of 60 to 0.741 ng/mL. Choose the range that fits your sample concentrations most appropriately.

The ANP Standard contains an organic solvent. Pipette the standard up and down several times to wet the pipet tip before transfer to ensure that volumes are accurate.

1. Add 50 µL ANP Standard to one tube containing 450 µL 1X Assay Buffer and label as 180 ng/mL ANP.
2. Add 200 µL 1X Assay Buffer to each of 6 tubes labeled as follows: 60, 20, 6.67, 2.22, 0.741, and 0 ng/mL ANP.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. **Use the standards within 2 hours of preparation**.

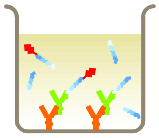


Perform ELISA (Total assay time: 1.5 hours)

IMPORTANT! Perform a standard curve with each assay.

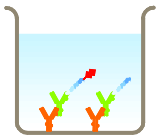
Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store desiccated at 2°C to 8°C for future use. The silica pack in the bag keeps the plate dry, and turns from blue to pink if the bag is not properly sealed.



Bind antigen

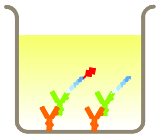
a. Add 50 µL of standards or samples (see "Prepare samples" on page 2) to the appropriate wells.
b. Add 75 µL of 1X Assay Buffer into wells for detecting non-specific binding (NSB).
c. Add 25 µL of ANP Conjugate to each well.
d. Add 25 µL of ANP Antibody to each well except NSB wells.
e. Tap the side of the plate to mix. Cover the plate with plate sealer and incubate for 1 hour at room temperature with shaking.
f. Thoroughly aspirate the solution and wash wells 4 times with 300 µL of 1X Wash Buffer.



Add chromogen

a. Add 100 µL TMB Substrate to each well. The substrate solution will begin to turn blue.
b. Incubate for 30 minutes at room temperature without shaking.

Note: TMB should not touch aluminum foil or other metals.



Add stop solution

Add 50 µL Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 10 minutes after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals lower than that of the highest standard in 1X Assay Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–180 ng/mL ANP.

Standard ANP (ng/mL)	Optical Density (450 nm)*
180	0.175
60	0.276
20	0.535
6.67	0.881
2.22	1.171
0.741	1.333
0	1.406

Note: The NSB gave a Mean OD value of 0.090.

Intra-assay precision

Samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	27.2	11.3	5.33
%CV	3.3	4.8	8.3

CV = Coefficient of Variation

Inter-assay precision

Samples were assayed in duplicates in 16 assay runs by five operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	26.4	10.9	4.9
%CV	8.7	9.2	8.3

CV = Coefficient of Variation

Performance characteristics, continued

Expected values

Multiple human plasma samples were tested in the EIA.

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.

Recovery

Recovery 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 Sample %	High Sample %	Expected Conc. (pg/mL)	Observed Conc. (ng/mL)	% Recovery
80	20	10.8	11.8	109.3
60	40	15.4	16.4	106.5
40	60	20.0	20.8	104.0
20	80	24.7	25.6	103.6
			Mean Recovery	**105.8%**

Interferents

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

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

Specificity

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 ß-ANP (1-28)	50
Human gamma-ANP	40
Rat ANF (18-28)	60
Atriopeptin II	5
BNP	<0.001

Sensitivity

The analytical sensitivity of ANP is 0.49 ng/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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