Datasheet for ABIN414774

NPPA ELISA Kit

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Overview

Quantity:	96 tests
Target:	NPPA
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	15.6-1000 pg/mL
Minimum Detection Limit:	15.6 pg/mL
Application:	ELISA

Product Details

Purpose:	The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of ANP in Serum,Plasma,Biological Fluids
Sample Type:	Cell Culture Supernatant, Cell Lysate, Plasma, Serum, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of Atrial Natriuretic Peptide (ANP). No significant cross-reactivity or interference between Atrial Natriuretic Peptide (ANP) and analogues was observed.
Cross-Reactivity (Details):	No significant cross-reactivity or interference between Atrial Natriuretic Peptide (ANP) and analogues was observed.
Sensitivity:	6.0 pg/mL

Product Details

Components:	• Pre-coated, ready to use 96-well strip plate • Plate sealer for 96 wells • Standard Diluent • Assay Diluent A • Assay Diluent B • Stop Solution • Standard • Detection Reagent A • Detection Reagent B • Substrate A • Wash Buffer (30 × concentrate) • Instruction manual
Material not included:	• Microplate reader with 450 nm filter. • Precision single or multi-channel pipettes and disposable tips. • Eppendorf Tubes for diluting samples. • Deionized or distilled water. • Absorbent paper for blotting the microtiter plate. • Container for Wash Solution

Target Details

Target:	NPPA
Alternative Name:	ANP (NPPA Products)

Application Details

Application Notes:	• Limited by the current condition and scientific technology, we cannot completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So there might be some qualitative and technical risks to use the kit. • The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available. • Kits from different batches may be a little different in detection range, sensitivity and color developing time. • Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer. • Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be covered tightly to prevent the evaporation and contamination of microorganism. • There may be some foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results. Do not remove microtiter plate from the

	storage bag until needed. • Wrong operations during the reagents preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results. A microplate plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. or greater at 450 ± 10nm wavelength is acceptable for use in absorbance measurement. Please read the instruction carefully and adjust the instrument prior to the experiment. • Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended. • Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different batches might arise from above factors, too. • Kits from different manufacturers for the same item might produce different results, since we have not compared our products with other manufacturers.
Comment:	Information on standard material: The standard might be recombinant protein or natural protein, that will depend on the specific kit. Moreover, the expression system is E.coli or yeast or mammal cell. There is 0.05% proclin 300 in the standard as preservative. Information on reagents: The stop solution used in the kit is sulfuric acid with concentration of 1 mol/L. And the wash solution is TBS. The standard diluent contains 0.02 % sodium azide, assay diluent A and assay diluent B contain 0.01% sodium azide. Some kits can contain is BSA in them. Information on antibodies: The provided antibodies and their host vary in different kits.
Sample Volume:	100 µL
Assay Time:	3 h
Plate:	Pre-coated
Protocol:	The test principle applied in this kit is Sandwich enzyme immunoassay. The microtiter plate provided in this kit has been pre-coated with an antibody specific to Atrial Natriuretic Peptide (ANP). Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to Atrial Natriuretic Peptide (ANP). Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain Atrial Natriuretic Peptide (ANP), biotin-

	conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of Atrial Natriuretic Peptide (ANP) in the samples is then determined by comparing the O.D. of the samples to the standard curve.
Reagent Preparation:	• Bring all kit components and samples to room temperature (18-25°C) before use. • **Standard** - Reconstitute the Standard with 1.0mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently(not to foam). The concentration of the standard in the stock solution is 5,000pg/mL. Please firstly dilute the stock solution to 1,000pg/mL and the diluted standard serves as the highest standard (1,000pg/mL). Then prepare 7 tubes containing 0.5mL Standard Diluent and use the diluted standard to produce a double dilution series. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 1,000pg/mL, 500pg/mL, 250pg/mL, 125pg/mL, 62.5pg/mL, 31.2pg/mL, 15.6pg/mL, and the last EP tubes with Standard Diluent is the blank as 0pg/mL. • **Assay Diluent A and Assay Diluent B** - Dilute 6mL of Assay Diluent A or B Concentrate(2×) with 6mL of deionized or distilled water to prepare 12 mL of Assay Diluent A or B. (In fact, more than 6mL Assay Diluent A and Assay Diluent B are contained in the bottles. Therefore, in every test, please precisely pipette required amount of Diluent and make double dilution in a new container. The prepared working dilution cannot be frozen.) • **Detection Reagent A and Detection Reagent B** - Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute to the working concentration with working Assay Diluent A or B, respectively (1:100). • **Wash Solution** - Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600 mL of Wash Solution (1×). • **TMB substrate** - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again. Note: • Making serial dilution in the wells directly is not permitted. • Prepare standard within 15 minutes before assay. Please do not dissolve the reagents at 37°C directly. • Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µL for once pipetting. • The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once. • If crystals have formed in the Wash Solution concentrate (30×), warm to room temperature and mix gently until the crystals are completely dissolved. • Contaminated water or container for reagent preparation will influence the detection result.
Sample Collection:	**Serum:** Allow samples to clot for two hours at room temperature or overnight at 4°C before

centrifugation for 20 minutes at approximately 1000 × g. Assay immediately or store samples in aliquot at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 × g within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Biological Fluids: Centrifuge samples for 20 minutes at 1000 × g. Remove particulates and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Sample Preparation:

Notes:

- The supplier is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
- Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts from certain chemicals.
- Due to the possibility of mismatching between antigen from other origin and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
- Influenced by the factors including cell viability, cell number or sampling time, samples from cell culture supernatant may not be detected by the kit.
- Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Assay Procedure:

- 1. Determine wells for diluted standard, blank and sample. Prepare 7 wells for standard, 1 well for blank. Add 100 µL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells. Cover with the Plate sealer. Incubate for 2 hours at 37 °C.
- 2. Remove the liquid of each well, don't wash.
- 3. Add 100 µL of Detection Reagent A working solution to each well. Incubate for 1 hour at 37 °C after covering it with the Plate sealer.
- 4. Aspirate the solution and wash with 350 µL of 1× Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1~2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent

paper. Totally wash 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.

- 5. Add 100 µL of Detection Reagent B working solution to each well. Incubate for 30 minutes at 37 °C after covering it with the Plate sealer.
- 6. Repeat the aspiration/wash process for total 5 times as conducted in step 4.
- 7. Add 90 µL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 15 - 25 minutes at 37 °C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.
- 8. Add 50 µL of Stop Solution to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
- 9. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450 nm immediately.

Note:

- 1. Assay preparation: Keep appropriate numbers of wells for 1 experiment and remove extra wells from microplate. Rest wells should be resealed and stored at -20 °C.
- 2. Samples or reagents addition: Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. To avoid cross-contamination, change pipette tips between additions of standards, samples, and reagents. Also, use separated reservoirs for each reagent.
- 3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents are added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be controlled.
- 4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and false elevated absorbance reading.
- 5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), if the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
- 6. TMB Substrate is easily contaminated. Please protect it from light.
- 7. The environment humidity which is less than 60 % might have some effects on the final performance, therefore, a humidifier is recommended to be used at that condition.

Calculation of Results:	Average the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. Create a standard curve on log-log graph paper, with ANP concentration on the y-axis and absorbance on the x-axis. Draw the best fit straight line

Application Details

	through the standard points and it can be determined by regression analysis. Using some plot software, for instance, curve expert 1.30, is also recommended. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. In order to make the calculation easier, we plot the O.D. value of the standard (X-axis) against the known concentration of the standard (Y-axis), although concentration is the independent variable and O.D. value is the dependent variable. However, the O.D. values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), plotting log of the data to establish standard curve for each test is recommended. Typical standard curve below is provided for reference only.
Assay Precision:	Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Atrial Natriuretic Peptide (ANP) were tested 20 times on one plate, respectively. Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Atrial Natriuretic Peptide (ANP) were tested on 3 different plates, 8 replicates in each plate. CV(%) = SD/meanX100 Intra-Assay: CV<10% Inter-Assay: CV<12%
Restrictions:	For Research Use only

Handling

Precaution of Use:	The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
Handling Advice:	The stability of kit is determined by the loss rate of activity. The loss rate of this kit is less than 5 % within the expiration date under appropriate storage condition. To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.
Storage:	4 °C
Storage Comment:	• For unopened kit: All the reagents should be kept according to the labels on vials. The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20 °C upon receipt while the others should be at 0 °C. • For opened kit: When the kit is opened, the remaining reagents still need to be stored according to the above storage condition. Besides, please return the unused wells to the foil

Handling

	pouch containing the desiccant pack, and reseal along entire edge of zip-seal. Note: It is highly recommended to use the remaining reagents within 1 month provided this is within the expiration date of the kit. • For ELISA kit, 1 day storage at 30 °C can be considered as 2 months at 0 °C, which means 3 days at 30 °C equaling 6 months at 0 °C.
Expiry Date:	6 months

Publications

Product cited in:

Wang, Xu, Yuan, Li, Zhang, Zheng, Liu, Feng, Li, Li, Ma: "Sodium-glucose co-transporter-2 inhibitors suppress atrial natriuretic peptide secretion in patients with newly diagnosed Type 2 diabetes." in: **Diabetic medicine : a journal of the British Diabetic Association**, (2016) (PubMed).

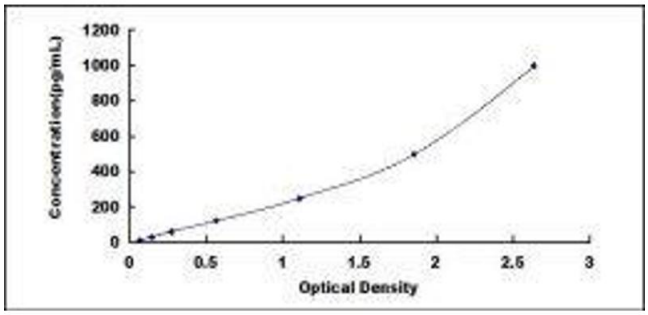
Chappell, Bruegger, Potzel, Jacob, Brettner, Vogeser, Conzen, Becker, Rehm: "Hypervolemia increases release of atrial natriuretic peptide and shedding of the endothelial glycocalyx." in: **Critical care**, Vol. 18, Issue 5, pp. 538, (2015) (PubMed).

Glahn, Riera Knorrenschild, Rhein, Haschemi Nassab, Gröschl, Heberlein, Muschler, Frieling, Bleich, Hillemacher: "Alcohol-induced changes in methylation status of individual CpG sites, and serum levels of vasopressin and atrial natriuretic peptide in alcohol-dependent patients during detoxification treatment." in: **European addiction research**, Vol. 20, Issue 3, pp. 143-50, (2014) (PubMed).

Glahn, Heberlein, Dürsteler-MacFarland, Lenz, Frieling, Gröschl, Wiesbeck, Kornhuber, Bönsch, Bleich, Hillemacher: "Atrial natriuretic peptide, arginine vasopressin peptide and cortisol serum levels in opiate-dependent patients." in: **Neuropsychobiology**, Vol. 67, Issue 2, pp. 111-5, (2013) (PubMed).

Damman, Ng Kam Chuen, MacFadyen, Lip, Gaze, Collinson, Hillege, van Oeveren, Voors, van Veldhuisen: "Volume status and diuretic therapy in systolic heart failure and the detection of early abnormalities in renal and tubular function." in: **Journal of the American College of Cardiology**, Vol. 57, Issue 22, pp. 2233-41, (2011) (PubMed).

There are more publications referencing this product on: Product page



ELISA

Image 1.