Datasheet for ABIN578766

NPPB ELISA Kit

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Overview

Quantity:	96 tests
Target:	NPPB
Reactivity:	Rat
Method Type:	Sandwich ELISA
Detection Range:	31.2-2000 pg/mL
Minimum Detection Limit:	31.2 pg/mL
Application:	ELISA

Product Details

Purpose:	This immunoassay kit allows for the in vitro quantitative determination of rat brain natriuretic peptide,BNP concentrations in serum, plasma and other biological fluids.
Sample Type:	Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay recognizes recombinant and natural rat BNP.
Cross-Reactivity (Details):	No significant cross-reactivity or interference was observed.
Sensitivity:	The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest detectable concentration that could be differentiated from zero.
Characteristics:	Rattus norvegicus,Rat,Natriuretic peptides B,Gamma-brain natriuretic peptide,Iso-ANP,Nppb
Components:	Reagent (Quantity): Assay plate (1), Standard (2), Sample Diluent (1 × 20ml), Assay Diluent A

Product Details

	(1x10ml), Assay Diluent B (1x10ml), Detection Reagent A (1 × 120µl), Detection Reagent B (1 × 120µl), Wash Buffer (25 x concentrate) (1 × 30ml), Substrate (1x10ml), Stop Solution (1x10ml), Plate sealer for 96 wells (5), Instruction (1)
Material not included:	Luminometer. Pipettes and pipette tips. EP tube Deionized or distilled water.

Target Details

Target:	NPPB
Alternative Name:	Nppb (NPPB Products)
Background:	Brain natriuretic peptide (BNP, also known as B-type natriuretic peptide or ",GC-B",) is a natriuretic hormone that is similar to ANP. It is a 32-amino-acid polypeptide secreted by the ventricles of the heart in response to excessive stretching of myocytes (heart muscles cells). Cardiac natriuretic hormones (CNHs) are a family of related peptides, including atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and other peptides derived from the N-terminal portion of the proANP and proBNP peptide chains. Brain natriuretic peptide (also known as B-type natriuretic peptide or ",GC-B",) is a 32 amino acid polypeptide secreted by the ventricles of the heart in response to excessive stretching of myocytes (heart muscles cells) in the ventricles. At the time of release, a co-secreted 76 amino acid N-terminal fragment (NT-proBNP) is also released with BNP. BNP binds to and activates NPRA in a similar fashion to atrial natriuretic peptide (ANP) but with 10-fold lower affinity. The biological half-life of BNP, however, is twice as long as that of ANP. Both ANP and BNP have limited ability to bind and activate NPRB. Brain natriuretic peptide was originally identified in extracts of porcine brain, but in humans it is produced mainly in the cardiac ventricles. Physiologic actions of BNP and ANP include decrease in systemic vascular resistance and central venous pressure as well as an increase in natriuresis. Thus, the resulting effect of these peptides is a decrease in cardiac output and a decrease in blood volume. BNP is a valuable marker in heart failure and its therapy, for example cardiac resynchronization therapy.
Gene ID:	3148
Pathways:	Hormone Activity

Application Details

Sample Volume:	100 µL
Plate:	Pre-coated

Application Details

Protocol:	The microtiter plate provided in this kit has been pre-coated with an antibody specific to BNP. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for BNP. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that 2 contain BNP, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm 2 nm. The concentration of BNP in the samples is then determined by comparing the O.D. of the samples to the standard curve.
Reagent Preparation:	Bring all reagents to room temperature before use. Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer. Standard - Reconstitute the Standard with 1.0 ml of Sample Diluent. This reconstitution produces a stock solution of 10,000 pg/mL. Allow the standard to sit for about 10 minutes with gentle agitation prior to making serial dilutions (Making serial dilution in the wells directly is not permitted). The undiluted standard serves as the highest standard (10,000 pg/mL). The Sample Diluent serves as the zero standard (0 pg/mL). 3 pg/mL 10,000 5,000 2,500 1,250 625 312 156 0 Detection Reagent A and B - Dilute to the working concentration using Assay Diluent A or B (1:100), respectively.
Sample Collection:	Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissue was rinsed with 1X PBS to remove excess blood, homogenized in 5~10 mL of 1X PBS and stored overnight at ≤ -20 °C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g. Remove the supernate and assay immediately or aliquot and store at ≤ -20 °C. Other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20 or -80 . Avoid repeated freeze-thaw cycles. Note: Tissue homogenates and other biological fluids to be used within 7 days may be stored at 2-8, otherwise samples must stored at -20 (1 month) or -80 (2 months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay slowly bring samples to room temperature.
Assay Procedure:	Allow all reagents to reach room temperature (Please do not dissolve the reagents at 37 directly.). All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be resealed and stored at 4 until the kits expiry date. Prepare all reagents, working standards and samples as directed in the previous sections. 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.

1. Add 100 of Standard, Blank, or Sample per well. Cover with the Plate sealer. Incubate for two hours at 37 .

2. Remove the liquid of each well, don't wash.

3. Add 100 µl of Detection Reagent A working solution to each well. Cover with the Plate sealer. Incubate for 1 hour at 37 . Detection Reagent A working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.

4. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (approximately 400 µl) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

5. Add 100 µl of Detection Reagent B working solution to each well. Cover with a new Plate sealer. Incubate for one hour at 37 .

6. Repeat the aspiration/wash process for five 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 - 30 minutes at 37 . Protect from light.

8. Add 50 µl of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

9. Determine the optical density of each well at once, using a microplate reader set to 450 nm.

4.

Important Note:1. Absorbance is a function of the incubation time. Therefore, prior to starting the assay it is recommended that all reagents should be freshly prepared prior to use and all required strip-wells are secured in the microtiter frame. This will ensure equal elapsed time for each pipetting step, without interruption.

2. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. The reconstituted Standards can be used only once. This assay requires pipetting of small volumes. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10µl for once pipetting.

3. 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 have been added to the well strips, DO NOT let the strips DRY at any time during the assay.

4. For each step in the procedure, total dispensing time for addition of reagents to the assay

Application Details

	plate should not exceed 10 minutes. 5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent. 6. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. 7. Duplication of all standards and specimens, although not required, is recommended. 8. Substrate Solution is easily contaminated. Please protect it from light.
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 by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an 5 alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the BNP concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. It is recommended to use some related software to do this calculation, such as curve expert 13.0. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
Restrictions:	For Research Use only

Handling

Handling Advice:	1. The kit should not be used beyond the expiration date on the kit label. 2. Do not mix or substitute reagents with those from other lots or sources. 3. If samples generate values higher than the highest standard, further dilute the samples and repeat the assay. Any variation in standard diluent, operator, pipetting technique, washing technique,incubation time or temperature, and kit age can cause variation in binding. 4. This assay is designed to eliminate interference by soluble receptors, ligands, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded. 5. Limited by the current condition and scientific technology, we can't 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.
Storage:	4 °C/-20 °C

Handling

Storage Comment:	The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20 °C upon being received. The other reagents can be stored at 4 °C.

