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FastELISA™ NPPB (Human) ELISA Kit

Catalog Number KA7037

1 Kit

Version: 01

Intended for research use only

www.abnova.com

Table of Contents

Introduction

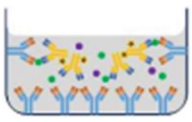
Intended Use

FastELISA™ NPPB (Human) ELISA Kit applies to the in vitro quantitative determination of Human NPPB (Brain natriuretic peptide, BNP) concentrations in serum, plasma, etc.

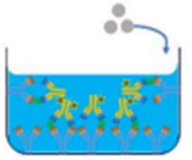
Principle of the Assay

FastELISA™ NPPB (Human) ELISA Kit uses the Sandwich-ELISA principle. The microtiter plate provided in this kit has been pre-coated with an antibody specific to Human NPPB, and the Human NPPB standard plate wells that pre-coated using protein-related techniques are provided separately. Standard/Sample Diluent Buffer or samples are added to the appropriate microtiter plate wells, then added a HRP-conjugated antibody specific to Human NPPB. After TMB substrate solution is added, only those wells that contain Human NPPB and HRP-conjugated antibody 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 450 ± 10nm. The concentration of Human NPPB in the samples is then determined by comparing the OD of the samples to the standard curve.

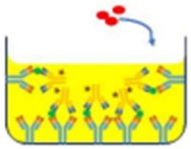
Assay Procedure Summary



1. After the kit is equilibrated at room temperature, add 50 µL Standard/Sample Diluent Buffer to each Standard well, and add 50 µL sample to the sample well. Immediately add 50 µL 1× HRP Conjugate Antibody Working Solution to each well, and incubate at 37°C on Microplate oscillator for 60 minutes.



2. Discard the liquid in the plate.
   Add 200 µL 1× Wash Buffer to each well, and wash the plate 5 times.
   After pat it dry against clean absorbent paper, add 90 µL TMB Substrate Solution to each well, and incubate at 37°C for 20 minutes in the dark.



3. Add 50 µL Stop Solution to each well, shake plate on a plate shaker for 1 minute to mix.
   Record the OD at 450 nm immediately and calculate the results.

General Information

Materials Supplied

List of components

Component	Amount	Storage condition
Pre-Coated Microplate	8 x 12 strips	4°C / -20°C
Standard Microplate	8 x 2 strips	4°C / -20°C
HRP Conjugate Antibody (100x)	70 µL	4°C / -20°C (store in the dark)
Standard/Sample Diluent Buffer	24 mL	4°C / -20°C
HRP Conjugate Diluent	10 mL	4°C / -20°C
Wash Buffer (25x)	24 mL	4°C / -20°C
TMB Substrate Solution	12 mL	4°C / -20°C (store in the dark)
Stop Reagent	7 mL	4°C / -20°C
Plate Covers	2 pcs	4°C / -20°C

Materials Required but not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 ± 10 nm
- ✓ High-speed centrifuge
- ✓ Electro-heating standing-temperature cultivator and Microplate oscillator
- ✓ Absorbent paper
- ✓ Double distilled water or deionized water
- ✓ Single or multi-channel pipettes with high precision and disposable tips
- ✓ Precision pipettes to deliver 2 µL to 1 mL volumes

Storage Instruction

If the kit is not opened, store the whole kit at 4°C (short-term storage, valid for 6 months); -20°C (long-term storage, valid for 1 year). Avoid repeated freeze-thaw cycles.
If the whole kit is stored at -20°C, place the kit at 4°C the day before the experiment.
If the kit is opened, store Standard Microplate at -20°C, the rest reagents at 4°C.
If the kit is not used up in 1 week, please store Standard Microplate, Pre-Coated Microplate and HRP Conjugate Antibody at -20°C, the rest reagents at 4°C, please used up within 6 months.

Precautions for Use

- ✓ This kit is only used for lab research and development and should not be used for human or animals.
- ✓ Reagents should be regarded as hazardous substances and should be handled carefully and correctly.
- ✓ Gloves, lab coats, and goggles should always be worn to avoid skin and eyes coming into contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.
- ✓ Do not use the kit beyond the expiration date.
- ✓ After receive the kit, please store the reagents according to the instructions. The plates can be disassembled to single strips. Please use it in batches on demand.
- ✓ After opening the package, please check that all components are complete.
- ✓ The cap must be tightened to prevent evaporation and microbial contamination. The reagents volume is slightly more than the volume marked on labels, please use accurate measuring equipment and do not pour directly into the vial.
- ✓ All kit components have been formulated and quality control tested to function successfully. Do not mix or substitute reagents or materials from other kits, detection effect of the kit will not be guaranteed if utilized separately or substituted.
- ✓ The test tubes, pipette tips and reagents used in the experiment are all disposable and are strictly prohibited from being reused; otherwise, the experiment results will be affected. Kit reagents of different batches cannot be mixed (except TMB, Washing Buffer and Stop Reagent).
- ✓ Bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.

Assay Protocol

Reagent Preparation

Bring all kit components and samples to room temperature (18-25°C) before use. Make sure all components are dissolved and mixed well before using the kit. If the kit will not be used up in 1 time, please only take out strips and reagents for present experiment, and save the remaining strips and reagents as specified.

✓ Wash Buffer (25x):

If crystals form in the concentrate (25×), it is a normal phenomenon. Heat it to room temperature (the heating temperature should not exceed 40°C), gently mix until crystals are completely dissolved.

Dilute the 25× Wash Buffer into 1× Wash Buffer with double-distilled Water.

✓ HRP Conjugate Antibody (100x):

HRP Conjugate Antibody is small in volume and may be scattered in various parts of the tube during transportation. Briefly spin or centrifuge 1000 × g for 1 minutes the stock HRP Conjugate Antibody before use. Before the experiment, the dosage required for the experiment (50 µL/ well, the actual configured total amount must be 50-100µL greater than the calculated value) was calculated, Dilute HRP Conjugate Antibody to the working concentration 100-fold with HRP Conjugate Diluent.

The dilution principle is to take 1 µL concentrated HRP conjugated antibody and add it to 99 µL HRP Conjugate Diluent and mix well.

Please configure the HRP Conjugate Antibody Working Solution according to the required amount and use the corresponding Dilution Solution. Cannot be mixed used.

✓ TMB Substrate Solution:

Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Notes:

Prior to use, equilibrate all materials and prepared reagents to room temperature and mix all reagents thoroughly while taking care not to create any foam within the vials.

Sample Preparation

✓ Serum:

Samples should be collected into a serum separator tube. After clotting for 2 hours at room temperature or overnight at 4°C, and then centrifuging at 1000 × g for 20 minutes. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

✓ Plasma:

Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples at 1000 × g and 2-8°C for 15 minutes within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

✓ Tissue homogenates:

The preparation of tissue homogenates will vary depending upon tissue type.

1. Rinse the tissues in pre-cooled PBS to completely remove excess blood, and weigh them before homogenization.
2. Mince the tissues to small pieces and homogenized them in fresh lysis buffer (different lysis buffer needs to be chosen based on subcellular location of the target protein) (PBS can be used as the lysis buffer of most tissues) (w:v = 1:9, e.g. 900 µL lysis buffer is added in 100 mg tissue sample) with a glass homogenizer on ice (micro tissue grinders, too).
3. Ultrasound the obtained suspension with an ultrasonic cell disrupter until the solution is clear.
4. Then, centrifuge the homogenates for 5 minutes at 10000 × g and collect the supernatant and assay immediately or store in aliquots at ≤ -20°C.

Notes: Tissue homogenates are recommended to be tested for protein concentration at the same time to obtain a more accurate concentration of the test substance per mg of protein.

✓ Cell lysates:

Cells need to be lysed before assaying according to the following directions.

1. Adherent cells should be washed by pre-cooled PBS gently, and then be detached with trypsin, and collect them by centrifugation at 1000 × g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells 3 times in pre-cooled PBS.
3. Then, resuspend the cells in fresh lysis buffer with concentration of 10^7 cells/mL. If it is necessary, the cells could be subjected to ultrasonication until the solution is clear.
4. Centrifuge at 1500 × g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or store in aliquots at ≤ -20°C.

✓ Urine:

Collect the first urine of the day (mid-stream) and discharge it directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

✓ Saliva:

Collect saliva using a collection device or equivalent. Centrifuge samples at 1000 × g at 2-8°C for 15 minutes. Remove particulates and assay immediately or store samples in aliquot at ≤ -20°C. Avoid repeated freeze-thaw cycles.

✓ Feces:

Dry feces were collected as much as possible, weighing more than 50 mg. The feces were washed three times with PBS (w:v = 1:9, e.g. 900 μL lysis buffer is added in 100 mg feces), sonicated (or mashed) and centrifuged at 5000×g for 10 minutes, where the supernatant was collected for testing.

✓ Cerebrospinal fluid (CSF):

Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

✓ Cell culture supernatants and other biological fluids:

Centrifuge samples at 1000 × g for 20 minutes. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

Sample Dilution

Normal Human serum and plasma samples are recommended for original solution testing.
Samples are diluted with Standard & Sample Diluent according to pre-test or above suggestion.

Notes

1. *Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤ 1 month) or -80°C (≤ 2 months) to avoid loss of bioactivity and contamination. Avoid repeated freeze-thaw cycles.*
2. *The sample should be clear and transparent, and the suspended matter should be removed by centrifugation. Sample hemolysis will influence the result, so it should not be used.*
3. *When performing the assay, bring samples to room temperature.*
4. *The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.*
5. *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.*
6. *If the concentration of the test material in your sample is higher than that of the Standard product, please make the appropriate multiple dilutions according to the actual situation (it is recommended to do preliminary experiment to determine the dilution ratio.*

Assay Procedure

Before the experiment starts, all reagents should be balanced to room temperature, and all reagents should be prepared in advance. When diluting the reagent or sample, it is necessary to mix, and try to avoid foaming when mixing. If the sample concentration is too high, dilute it with a sample diluent to make the sample conform to

the detection range of the kit.

1. Place the labeled standard strip in the frame of the Microplate, add 50 µL Standard/Sample Diluent Buffer to each standard well, add 50 µL sample to the sample wells (if the sample needs to be diluted, please refer to the sample dilution suggestion), pay attention to no bubbles, add the sample to the bottom of the Microplate well when adding the sample, do not touch the wall of the well.

   Note 1: The standard strips are provided separately that contains white solid standard. Re-seal the unused standard slats and place them at -20°C for use as soon as possible. Place the standard slats upwards as far as possible. When opening the cover, check whether there is a solid standard on the plug. If so, the solid standard needs to be moved into the corresponding well with a pipette.

   Note 2: If the Standard/Sample Diluent Buffer is added to the standard well but the white solid standard on the wall is not dissolved, it is necessary to flush the diluent in the appropriate well with a pipette to completely dissolve the standard product.

2. Then, each well was immediately added with 50 µL HRP conjugate antibody working solution, cover the microplate with Plate Cover. Oscillate the Microplate with the oscillator at 500 rpm and incubated at 37°C for 60 minutes.

   Note 1: The tips don't touch the liquid in the wells when adding HRP conjugate antibody working solution.

   Note 2: When incubating antigen and HRP conjugate antibody, it is necessary to use a micro-plate oscillator to oscillate the Microplate. If the plate is not oscillated, the reaction will be inadequate, and the OD value will decrease overall. Other horizontal oscillators should adjust their own speed to ensure that the solution per well did not exceed half height of the wells and could be mixed. Too much oscillation would cause the background to rise.

3. Discard the liquid in the wells and wash the plate 5 times. Wash each well with 200 µL of washing solution, soak for 1-2 minutes each time, and shake off the liquid in the plate (or wash the plate with a plate washer). After the last wash, pat the plate dry on absorbent paper.
4. Add 90 µL of TMB Substrate Solution to each well. Cover with a new Plate Cover. Incubate for 20 minutes at 37°C (Don't exceed 30 minutes) in the dark. The liquid will turn blue by the addition of TMB Substrate Solution. Preheat the Microplate Reader for about 15 minutes before OD measurement.

   Note: Dispense the TMB Substrate Solution within 15 minutes following the washing of the microtiter plate.

5. Add 50 µL of Stop Reagent to each well. The liquid will turn yellow by the addition of Stop Reagent. 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. The insertion order of the Stop Reagent should be the same as that of the TMB Substrate Solution.
6. Wipe off 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.

Data Analysis

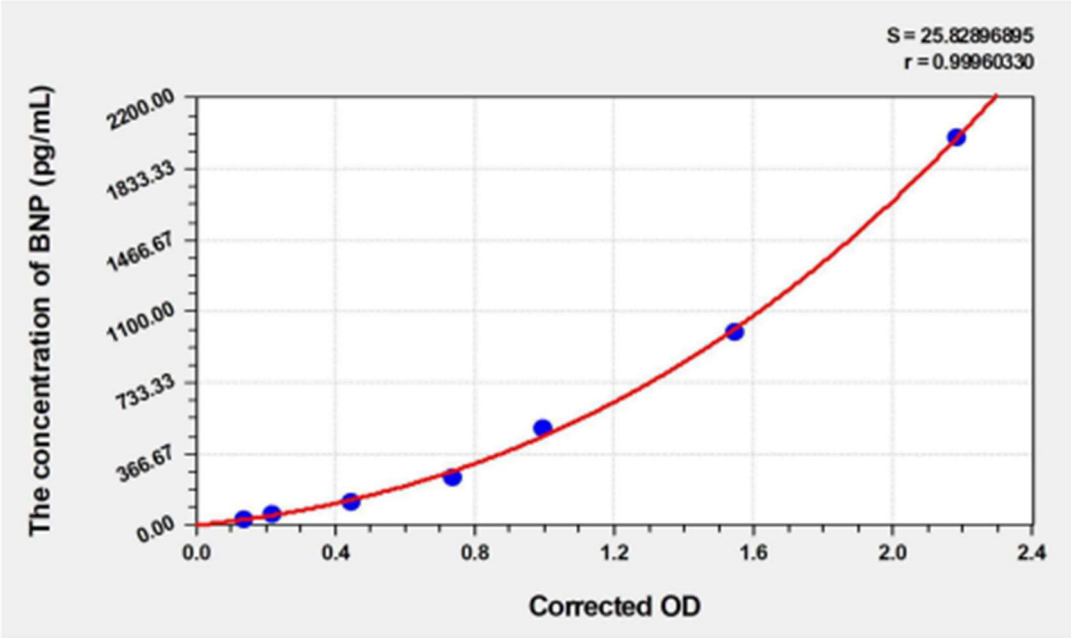
Calculation of Results

Average the duplicate readings for each Standard, Control, and Samples and subtract the average zero Standard optical density. Construct a Standard curve with the Human NPPB concentration on the y-axis and absorbance on the x-axis, and draw a best fit curve through the points on the graph. If samples have been diluted, the concentration read from the Standard curve must be multiplied by the dilution factor. Using some plot software, for instance, curve expert.

✓ Typical Data

− Standard curve:

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data is provided below for reference only



(pg/mL)	2,000	1,000	500	250	125	62.5	31.25	0
OD	2.266	1.633	1.088	0.826	0.539	0.313	0.235	0.096
Corrected OD	2.17	1.537	0.992	0.73	0.443	0.217	0.139	-

− Sensitivity: 6.8 pg/mL

− Detection range: 31.25-2,000 pg/mL

− Specificity: This assay has high sensitivity and excellent specificity for detection of Human NPPB. No significant cross-reactivity or interference between Human NPPB and analogues was observed.

− Precision:

Intra-assay Precision (Precision within an assay): CV% < 8%

Three samples of known concentration were tested twenty times on 1 plate to assess intra-assay precision.

Inter-assay Precision (precision between assays)：CV% < 10%

Three samples of known concentration were tested in forty separate assays to assess inter-assay precision.

− Recovery: Matrices listed below were spiked with certain level of recombinant Human NPPB and the recovery rates were calculated by comparing the measured value to the expected amount of Human NPPB in samples.

Matrix	**Recovery Range (%)**	**Average Recovery (%)**
Serum (n=5)	88-102	95
EDTA plasma (n=5)	80-93	86
Heparin plasma (n=5)	87-97	90

− Linearity: The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Human NPPB and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	Serum (n=5)	EDTA plasma (n=5)	Heparin plasma (n=5)
1:2	87-98%	86-102%	98-105%
1:4	85-92%	87-96%	92-101%
1:8	79-96%	89-98%	85-105%
1:16	89-100%	83-103%	87-98%

Resources

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H