# Nppb (Rat) ELISA Kit

Catalog Number KA0979

96 assays

Version: 09

Intended for research use only

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## Introduction

### Background

Natriuretic peptides (ANP, BNP and CNP) comprise a family of structurally related peptides, which are derived from three different genes and share a 17-amino acid internal ring (1).

### Principle of the Assay

The Nppb (Rat) ELISA kit is designed for detection of rat BNP-45 in plasma, serum, tissue extract, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures rat BNP45 in less than 5 hours. A polyclonal antibody specific for rat BNP-45 has been pre-coated onto a 96-well microplate with removable strips. The rat BNP-45 in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for rat BNP-45, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

## General Information

### Materials Supplied

List of component

| Component | Amount |
|---|---|
| Rat BNP-45 Microplate: A 96 well polystyrene microplate coated with a polyclonal antibody against rat BNP-45. | 96 (8x12) wells |
| Sealing Tapes: Pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay. | 3 slices |
| Rat BNP45 Standard: Rat BNP-45 in a buffered protein base (lyophilized). | 8 ng |
| Biotinylated Rat BNP-45 Antibody (50x): A 50-fold biotinylated polyclonal antibody against rat BNP-45. | 120 µL |
| MIX Diluent Concentrate (10x): A 10-fold buffered protein base. | 30 mL |
| Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant. | 30 mL x 2 |
| Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate. | 80 µL |
| Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine. | 8 mL |
| Stop Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction. | 12 mL |

### Storage Instruction

- ✓ Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- ✓ Store SP Conjugate and Biotinylated Antibody at -20°C.
- ✓ Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop solution and Chromogen substrate at 2-8°C.
- ✓ Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- ✓ Diluent (1x) may be stored for up to 30 days at 2-8°C.
- ✓ Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

### Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Pipettes (1-20 µL, 20-200 µL, 200-1000 µL and multiple channels).
- ✓ Deionized or distilled reagent grade water

**Precautions for Use**

- ✓ This product is for Research Use Only and is not intended for use in diagnostic procedures.
- ✓ Prepare all reagents (working diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.
- ✓ Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
- ✓ Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.
- ✓ The Stop Solution is an acidic solution.
- ✓ The kit should not be used beyond the expiration date.

## Assay Protocol

### Reagent Preparation

Freshly dilute all reagents and bring all reagents to room temperature before use.

- ✓ MIX Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2- 8°C.
- ✓ Rat BNP-45 Standard: Reconstitute the 8 ng of Rat BNP-45 Standard with 4 mL of MIX Diluent to generate a 2 ng/mL standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard stock solution (2 ng/mL) 1:2 with MIX Diluent to generate 1, 0.5, 0.25, 0.125, 0.0625, and 0.0313 ng/mL solutions. MIX Diluent serves as the zero standard (0 ng/mL). Any remaining solution should be frozen at -20°C and used within 30 days.

| Standard Point | Dilution | [Rat BNP-45] (ng/mL) |
|---|---|---|
| P1 | 1 part Standard (2 ng/mL) + 1 part MIX Diluent | 1.0 |
| P2 | 1 part P1 + 1 part MIX Diluent | 0.5 |
| P3 | 1 part P2 + 1 part MIX Diluent | 0.25 |
| P4 | 1 part P3 + 1 part MIX Diluent | 0.125 |
| P5 | 1 part P4 + 1 part MIX Diluent | 0.0625 |
| P6 | 1 part P5 + 1 part MIX Diluent | 0.0313 |
| P7 | MIX Diluent | 0.0000 |

- ✓ Biotinylated Rat BNP-45 Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- ✓ Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- ✓ SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

### Sample Preparation

- ✓ Plasma: Collect plasma using a final concentration of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and assay undiluted plasma for medium and high level of BNP-45. Samples are recommended for use at 1x or diluted 2x-10x. Depending on application needs, user should determine proper dilutions. Samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- ✓ Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes. Remove serum and perform the assay for medium and high level of BNP-45. Samples are recommended for use at 1x or diluted 2x-10x. Depending on application needs,

user should determine proper dilutions. Samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

- ✓ Cell Culture Supernatants: Centrifuge cell culture media at 3000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.
- ✓ Tissue: Extract tissue samples with 0.1 M phosphate-buffered saline (pH7.4) containing 1% Triton X-100 and centrifuge at 14000 x g for 20 min. Collect the supernatant, measure the protein concentration, and assay. Freeze the remaining extract at -20°C or below.

**Assay Procedure**

1. Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
3. Add 50 µL of Rat BNP-45 Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
4. Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µL of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
5. Add 50 µL of Biotinylated Rat BNP-45 Antibody to each well and incubate for 2 hours.
6. Wash the microplate as described above.
7. Add 50 µL of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
8. Wash the microplate as described above.
9. Add 50 µL of Chromogen Substrate per well and incubate for 8 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
10. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow.
11. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

✓ Assay Summary

1. Add 50 µL of Standard or Sample per well. Incubate 2 hours.
2. Wash, then add 50 µL of Biotinylated Antibody per well. Incubate 2 hours.
3. Wash, then add 50 µL of SP Conjugate per well. Incubate 30 minutes.
4. Wash, then add 50 µL of Chromogen Substrate per well. Incubate 8 minutes.
5. Add 50 µL of Stop Solution per well. Read at 450 nm immediately.

## Data Analysis

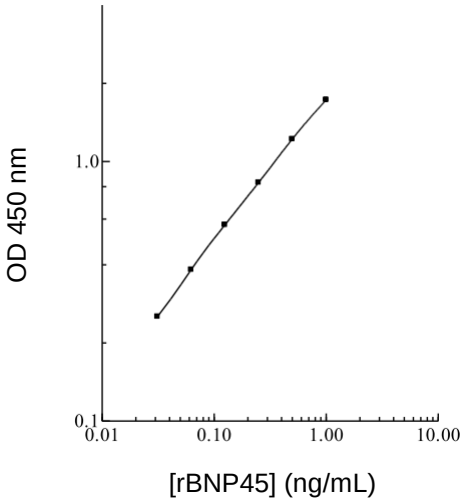
### Calculation of Results

- ✓ Calculate the mean value of the triplicate readings for each standard and sample.
- ✓ To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using 4-parameter or log-log logistic curve-fit.
- ✓ Determine the unknown sample concentration from the standard curve and multiply the value by the dilution factor.
- ✓ The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

| Standard Point | ng/mL | OD | Average OD |
|---|---|---|---|
| P1 | 1.0 | 1.749<br>1.711 | 1.730 |
| P2 | 0.5 | 1.215<br>1.227 | 1.221 |
| P3 | 0.25 | 0.867<br>0.888 | 0.877 |
| P4 | 0.125 | 0.613<br>0.605 | 0.609 |
| P5 | 0.0625 | 0.392<br>0.373 | 0.383 |
| P6 | 0.0313 | 0.256<br>0.250 | 0.253 |
| P7 | 0.0000 | 0.128<br>0.132 | 0.130 |

✓ Standard Curve

The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.




**Performance Characteristics**

✓ The minimum detectable dose of rat BNP-45 as calculated by 2SD from the mean of a zero standard was established to be 0.025 ng/mL.

✓ Intra-assay precision was determined by testing replicates of three plasma samples in one assay.

✓ Inter-assay precision was determined by testing three plasma samples in twenty assays.

| | Intra-Assay Precision | | | Inter-Assay Precision | | |
|---|---|---|---|---|---|---|
| Sample | 1 | 2 | 3 | 1 | 2 | 3 |
| n | 20 | 20 | 20 | 20 | 20 | 20 |
| CV (%) | 4.8% | 4.5% | 4.9% | 8.6% | 8.8% | 9.2% |
| Average (CV%) | 4.7% | | | 8.9% | | |

## Resources

**Troubleshooting**

| Issue | Causes | Course of Action |
|---|---|---|
| Low Precision | Use of expired components | • Check the expiration date listed before use.<br>• Do not interchange components from different lots. |
| | Improper wash step | • Check that the correct wash buffer is being used.<br>• Check that all wells are dry after aspiration.<br>• Check that the microplate washer is dispensing properly.<br>• If washing by pipette, check for proper pipetting technique. |
| | Splashing of reagents while loading wells | • Pipette properly in a controlled and careful manner. |
| | Inconsistent volumes loaded into wells | • Pipette properly in a controlled and careful manner.<br>• Check pipette calibration.<br>• Check pipette for proper performance. |
| | Insufficient mixing of reagent dilutions | • Thoroughly agitate the lyophilized components after reconstitution.<br>• Thoroughly mix dilutions. |
| | Improperly sealed microplate | • Check the microplate pouch for proper sealing.<br>• Check that the microplate pouch has no punctures.<br>• Check that three desiccants are inside the microplate pouch prior to sealing. |
| Unexpectedly Low or High Signal Intensity | Microplate was left unattended between steps | • Each step of the procedure should be performed uninterrupted. |
| | Omission of step | • Consult the provided procedure for complete list of steps. |
| | Step performed in incorrect order | • Consult the provided procedure for the correct order. |
| | Insufficient amount of reagents added to wells | • Check pipette calibration.<br>• Check pipette for proper performance. |
| | Wash step was skipped | • Consult the provided procedure for all wash steps. |
| | Improper wash buffer | • Check that the correct wash buffer is being used. |
| | Improper reagent preparation | • Consult reagent preparation section for the correct dilutions of all reagents. |
| | Insufficient or prolonged incubation periods | • Consult the provided procedure for correct incubation time. |

| | | |
|---|---|---|
| Deficient Standard Curve Fit | Non-optimal sample dilution | • Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay.<br>• Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay.<br>• User should determine the optimal dilution factor for samples. |
| | Contamination of reagents | • A new tip must be used for each addition of different samples or reagents during the assay procedure. |
| | Contents of wells evaporate | • Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature. |
| | Improper pipetting | • Pipette properly in a controlled and careful manner.<br>• Check pipette calibration.<br>• Check pipette for proper performance. |
| | Insufficient mixing of reagent dilutions | • Thoroughly agitate the lyophilized components after reconstitution.<br>• Thoroughly mix dilutions. |

**References**

1. Wiedemann K, Jahn H, Kellner M Exp Clin Endocrinol Diabetes 2000;108(1):5-13

**Plate Layout**

| | | | | | | | | |
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| | A | B | C | D | E | F | G | H |