Product Information

Brain Natriuretic Peptide EIA Kit

for serum, plasma, culture supernatant, and cell lysates

RAB0386

Storage: −20 °C

Product Description

The Brain Natriuretic Peptide (BNP) Enzyme Immunoassay (EIA) Kit is an *in vitro* quantitative assay for detecting BNP peptide based on the principle of competitive enzyme immunoassay. In this assay, a biotinylated BNP peptide is spiked into the samples and standards. The samples and standards are then added to the plate, where the biotinylated BNP peptide competes with endogenous (unlabeled) BNP for binding to the anti-BNP antibody. After a wash step, any bound biotinylated BNP then interacts with horseradish peroxidase (HRP)-streptavidin, which catalyzes a color development reaction. The intensity of the colorimetric signal is directly proportional to the amount of captured biotinylated BNP peptide and inversely proportional to the amount of endogenous BNP in the standard or samples. A standard curve of known concentration of BNP peptide can be established and the concentration of BNP peptide in the samples can be calculated accordingly.

Storage

The entire kit may be stored at -20°C to -80°C for up to 6 months from the date of shipment. For extended storage, it is recommended to store at -80°C. Avoid repeated freeze-thaw cycles.

Components

1. 96-well plate coated with secondary antibody (Item A)-RAB0386A: 96-wells (12 strips x 8 wells) coated with secondary antibody. Stable in storage 1 month at 4 °C once opened (Return unused wells to the pouch containing desiccant pack, reseal along entire edge).
2. 20x Wash Buffer (Item B)-RABWASH3: 25 mL. Stable in storage 1 month at 4 °C once opened.
3. EIA Brain Natriuretic Peptide standard, Lyophilized (Item C)-RAB0386C: 2 vials. Do not store and reuse.
4. Anti-Brain Natriuretic Peptide Detection Antibody, Lyophilized (Item N)-RAB0386F: 2 vials. Do not store and reuse.
5. EIA Brain Natriuretic 5x Assay Diluent B (Item E)-RABDIL10: 15 mL of 5x concentrated buffer. Stable in storage 1 month at 4 °C once opened.
6. Biotinylated Brain Natriuretic Peptide, Lyophilized (Item F)-RAB0386G: 2 vials. Do not store and reuse.
7. HRP-streptavidin (Item G)-RABHRP3: 600 µL of 200x concentrated HRP-conjugated Streptavidin. Do not store and reuse.
8. Brain Natriuretic Peptide Positive Control Sample, Lyophilized (Item M)-RAB0386K: 1 vial. Do not store and reuse.
9. TMB Substrate solution (Item H)-RABTMB2: 12 mL of 3,3’,5,5’-tetramethylbenzidine (TMB) in buffered solution.
10. Stop Solution (Item I)-RABSTOP2: 8 mL of 0.2 M sulfuric acid.

Reagents and Equipment Required (Not Provided)

1. Microplate reader capable of measuring absorbance at 450nm.
2. Precision pipettes to deliver 2 µL to 1 mL volumes.
3. Adjustable 1-25 mL pipettes for reagent preparation.
4. 100 mL and 1-liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. SigmaPlot software (or other software which can perform four-parameter logistic regression models)
8. Tubes to prepare standard or sample dilutions.
9. Orbital shaker
10. Aluminum foil

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

1. Keep kit reagents on ice during reagent preparation steps. Equilibrate plate to room temperature before opening the sealed pouch.
2. 5x Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water.
3. Briefly centrifuge the anti-BNP antibody vial (Item N) and reconstitute with 55 µL of 1x Assay Diluent B to prepare the antibody concentrate. Pipette up and down to mix gently.
4. The antibody concentrate should then be diluted 100-fold with 1x Assay Diluent B. This is the anti-BNP antibody working solution, which will be used in Procedure, step 2.

   Note: The following steps may be done during the antibody incubation procedure (Procedure, step 2).
5. Briefly centrifuge the vial of Biotinylated BNP peptide (Item F) and reconstitute with 20 µL of water before use. Transfer the entire contents of the Item F vial into a tube containing 10 mL of 1x Assay Diluent B. This is the Working Stock of Item F. Pipette up and down to mix gently. The final concentration of biotinylated BNP will be 20 pg/mL.
   - (a) Second Dilution of Item F for Standards: Add 2 mL of Working Stock Item F to 2 mL of 1x Assay Diluent B. The final concentration of biotinylated BNP will be 10 pg/mL.
   - (b) Second Dilution of Item F for Positive Control: Add 100 µL of Working Stock Item F to 100 µL of the prepared Positive Control (Item M). (Preparation, step 7) The final concentration of biotinylated BNP will be 10 pg/mL.
   - (c) Second Dilution of Item F for samples: Add 125 µL of Working Stock Item F to 125 µL of prepared sample (Preparation, step 8). This is a 2-fold dilution of the sample. The final concentration of biotinylated BNP will be 10 pg/mL.
6. Preparation of Standards: Label 6 microtubes with the following concentrations: 1,000, 100, 10, 1, 0.1, and 0 pg/mL. Pipette 450 µL of biotinylated BNP item F working solution (Preparation, step 5a) into each tube, except for the 1,000 pg/mL (leave this one empty).

   Note: It is very important to make sure the concentration of biotinylated BNP is 10 pg/mL in all standards.
   - (a) Briefly centrifuge the vial of BNP Standard (Item C) and reconstitute with 10 µL of water. In the tube labeled 1,000 pg/mL, pipette 8 µL of Item C and 792 µL of 10 pg/mL biotinylated BNP working solution (Preparation, step 5a). This solution serves as the first standard (1000 pg/mL BNP standard, 10 pg/mL biotinylated BNP).
   - (b) To make the 100 pg/mL standard, pipette 50 µL of the 1000 pg/mL BNP standard into the tube labeled 100 pg/mL. Mix thoroughly.

- (c) Repeat this step with each successive concentration, preparing a dilution series as shown in Figure 1. Each time, use 450 µL of biotinylated BNP and 50 µL of the prior concentration until the 0.1 pg/mL is reached. Mix each tube thoroughly before the next transfer.
- The final tube (0 pg/mL BNP, 100 pg/mL biotinylated BNP) serves as the zero standard (or total binding).

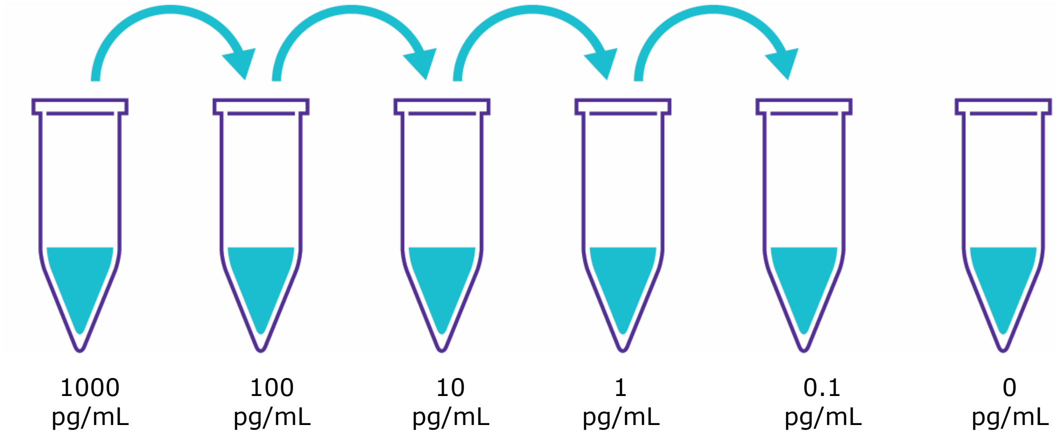


Figure 1: Dilution Series for Standards

7. Positive Control Preparation: Briefly centrifuge the positive control vial (Item M) and reconstitute with 100 µL of water. (Preparation, step 5b) This is a 2-fold dilution of the Positive Control. The final concentration of biotinylated BNP should still be 10 pg/mL. The Positive Control is a serum sample that serves as a system control to verify the kit components are working. The resulting OD will not be used in any calculations, if no positive competition is observed please contact Technical Services. The Positive Control may be diluted further if desired, but be sure the final concentration of biotinylated BNP is 10 pg/mL.
8. Sample Preparation: 2-fold dilution of sample (Preparation, step 5c). To perform a higher dilution of the sample, dilute the sample with 1x Assay Diluent B before performing (Preparation, step 5c).

   Example (to make a 4-fold dilution of sample): a. Dilute sample 2-fold (62.5 µL of sample plus 62.5 µL of 1x Assay Diluent B.). b. Perform step 5c (125 µL of working solution Item F plus 125 µL of sample prepared above). The total volume is 250 µL, enough for duplicate wells on the microplate. It is very important to make sure the final concentration of the biotinylated BNP is 10 pg/mL.

   Note: Optimal sample dilution factors should be determined empirically, reference for recommended dilution factors for serum: human=2x, mouse=8x, rat=2x.
9. If Item B (20x Wash Concentrate) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1x Wash Buffer.
10. Briefly centrifuge the HRP-Streptavidin vial (Item G) before use. Dilute the HRP-Streptavidin concentrate 200-fold with 1x Assay Diluent B.

Procedure

1. Keep kit reagents on ice during reagent preparation steps. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µL of anti-BNP antibody (Item N) (Preparation, step 4) to each well. Incubate for 1.5 hours at room temperature with gentle shaking (1-2 cycle/sec) or incubate overnight at 4 °C.
3. Discard the solution and wash wells 4 times with 1x Wash Solution Buffer (200-300 µL each). Washing may be done with a multichannel pipette or an automated plate washer. Complete removal of liquid at each step is essential to good assay performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µL of each standard (Preparation, step 6), Positive Control (Preparation, step 7), and sample (Preparation, step 8) in appropriate wells. Be sure to include a blank well (Assay Diluent only). Cover wells and incubate for 2.5 hours at room temperature with gentle shaking (1-2 cycles/sec) overnight or at 4 °C.

5. Discard the solution and wash 4 times as directed in Step 3.
6. Add 100 µL of prepared HRP-Streptavidin solution (Preparation, step 10) to each well. Incubate for 45 minutes at room temperature with gentle shaking. It is recommended that incubation time should not be shorter or longer than 45 minutes.
7. Discard the solution and wash 4 times as directed in Step 3.
8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking (1-2 cycles/sec).
9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.

Results

Calculations

Calculate the mean absorbance for each set of duplicate standards, controls, and samples, and subtract the blank optical density. Plot the standard curve using SigmaPlot software (or other software which can perform four-parameter logistic regression models), with standard concentration on the x-axis and percentage of absorbance (see calculation below) on the y-axis. Draw the best-fit curve through the standard points.

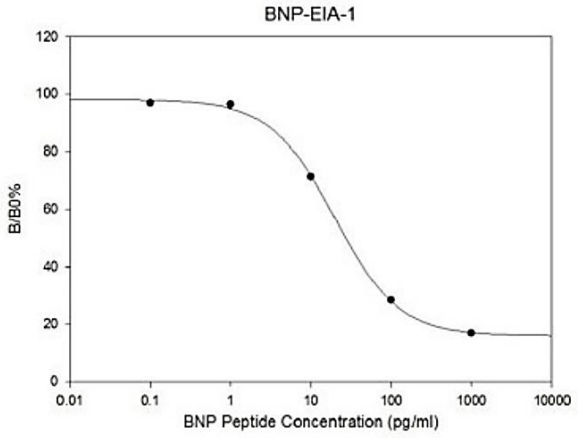
Percentage absorbance =

(B-blank OD)/B_0-blank OD)

where
B=OD of sample or standard
B_0=OD of zero standard (total binding)

Typical Data

Standard curve(s) is for demonstration only. Standard curve(s) must be run with each assay.



Product Profile

Sensitivity: The minimum detectable concentration of BNP is 1.66 pg/mL.

Detection Range:
0.1-1,000 pg/mL

Reproducibility:
Intra-Assay: CV <10%
Intra-Assay: CV <15%

Specificity

Cross Reactivity: This EIA kit shows no cross-reactivity with any of the adipokines tested: Ghrelin, Nesfatin, Angiotensin II, NPY and APC.

Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure a brief spin of Item C and dissolve the powder thoroughly with gentle mixing.
Low signal	Improper preparation of standard and/or biotinylated antibody	Briefly spin down vials before opening. Dissolve the powder thoroughly
	Too brief incubation times	Ensure sufficient incubation time; Procedure, step 2 may done overnight
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Air bubbles in wells	Remove bubbles in wells
	Inaccurate pipetting	Check pipettes
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the standard at <−20 °C reconstitution, others at 4 °C. Keep substrate solution protected from light
	Stop solution	Stop solution should be added to each well before measurement.

Notice

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