NPPB Chemi-Luminescent ELISA Kit (Dog) (OKCD05539)

Instructions for use

For the quantitative measurement of NPPB in Serum, plasma and other biological fluids

Lot to lot kit variations can occur. Refer to the manual provided with the kit.

This product is intended for research use only.

Table of Contents

1. Background

Principle

Aviva Systems Biology NPPB Chemi-Luminescent ELISA Kit (Dog) (OKCD05539) is based on standard sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific for NPPB has been pre-coated onto a 96-wellplate (12 x 8 Well Strips). Standards or test samples are added to the wells, incubated and removed. A biotinylated detector antibody specific for NPPB is added, incubated and followed by washing. Avidin-Peroxidase Conjugate is then added, incubated and unbound conjugate is washed away. An enzymatic reaction is produced through the addition of a luminol substrate which is catalyzed by the HRP to produce light emission. The light emission is read by a luminometer (or photo-multiplier equipped instrument) and the intensity of the emitted light is proportional to the amount of sample NPPB captured in well.

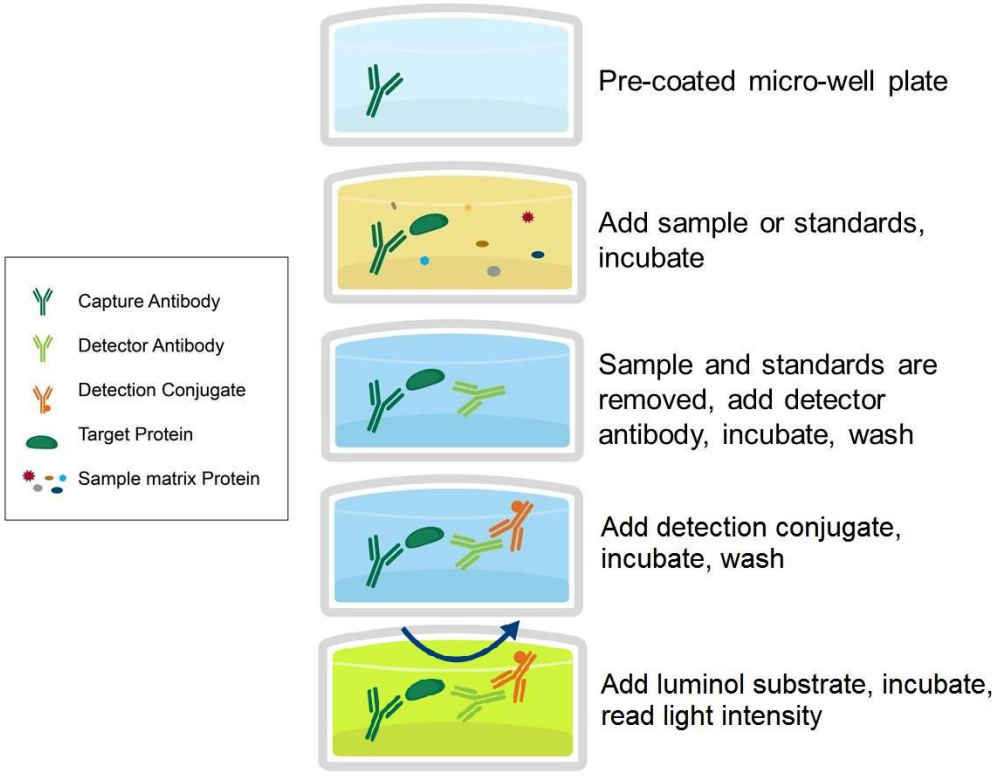
Target Background

Cardiac hormone which may function as a paracrine antifibrotic factor in the heart. Also plays a key role in cardiovascular homeostasis through natriuresis, diuresis, vasorelaxation, and inhibition of renin and aldosterone secretion. Specifically binds and stimulates the cGMP production of the NPR1 receptor. Binds the clearance receptor NPR3.

General Specifications

General Specifications	
Range	1.37-1,000pg/mL
LOD	< 0.55pg/mL (Derived by linear regression of OD_{450} of the Mean Blank + 2xSD)
Specificity	NPPB UniProt ID: P16859 GeneID: 487441 Target Alias: NT-Pro-BNP; ; N-BNP
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

2. Assay Summary



3. Storage and Stability

• Open kit immediately upon receipt. Store kit at -20°C for 6 months, noted exceptions below. Do not use past expiration date.

4. Kit Components

•The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
Anti-NPPB Microplate	96 Wells (12 x 8 Well strips)	-20°C for 6 months
NPPB Lyophilized Standard	2 x 10 ng	
100X Biotinylated NPPB Detector Antibody	1 x 120 µL	
100X Avidin-HRP Conjugate	1 x 120 µL	
Standard Diluent	1 x 20 mL	4°C for 6 months
Detector Antibody Diluent	1 x 12 mL	
Conjugate Diluent	1 x 12 mL	
30X Wash Buffer	1 x 20 mL	
100X Luminol Substrate	1 x 2 mL	
Substrate Diluent	1 x 10 mL	

5. Precautions

• Read instructions fully prior to beginning use of the assay kit.
• Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
• Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
• For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).
• Kit cannot be used beyond the expiration date on the label.

6. Required Materials Not Supplied

• Luminometer or photo-multiplier tube (PMT) equipped microplate reader capable of the following parameters: lag time 30.0 seconds, read time 1.0 seconds per well.
• Automated plate washer (optional).
• Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
• Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
• New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
• Absorbent paper or paper toweling.
• Distilled or deionized ultrapure water.
• 37°C Incubator (optional)

7. Technical Application Tips

• Do not mix or substitute components from other kits.
• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.
• Samples exhibiting light intensity measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.
• Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.
• Replicate wells are recommended for standards and samples.
• Cover microplate while incubating to prevent evaporation.
• Do not allow the microplate wells dry at any point during the assay procedure.
• Do not reuse tips or tube to prevent cross contamination.
• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.
• Completely remove of all liquids when washing to prevent cross contamination.
• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.
• Equilibrate all materials to ambient room temperature prior to use (standards exception).
• For optimal results for inter- and intra-assay consistency, equilibrate all materials to 37°C prior to performing assay (standards exception) and perform all incubations at 37°C.
• Pipetting less than 1 µL is not recommended for optimal assay accuracy.
• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.
• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.
• Samples containing precipitates, fibrin strands or bilirubin, or are hemolytic or lipemic might cause inaccurate results due to interfering factors.
• Luminol Substrate is easily contaminated and labile. Handle carefully and protect from light.

8. Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use immediately.

8.1 Dog NPPB Assay Standards

8.1.1 Prepare the NPPB standards no greater than 2 hours prior to performing experiment. Standards should be held on ice until use in the experiment.

8.1.2 Reconstitute one vial of the provided **10 ng Lyophilized Standard** for each experiment. Prepare a stock **10,000 pg/mL Standard** by reconstituting one tube of **Lyophilized Standard** as follows:

8.1.2.1 Gently spin or tap the vial at 6,000 – 10,000 rpm for 30 seconds to collect all material at the bottom.

8.1.2.2 Add 1 mL of **Standard Diluent** to the vial.

8.1.2.3 Seal the vial then mix gently and thoroughly.

8.1.2.4 Leave the vial at ambient temperature for 15 minutes.

8.1.3 Prepare a set of seven serially diluted standards as follows:

8.1.3.1 Label tubes with numbers 1 – 8.

8.1.3.2 Add 600 µL of **Standard Diluent** to Tube #'s 2 – 8.

8.1.3.3 Prepare a **2,000 pg/mL Standard #1** by adding 200 µL of **10,000 pg/mL Standard** to 800 µL of **Standard Diluent** in Tube #1. Mix gently and thoroughly.

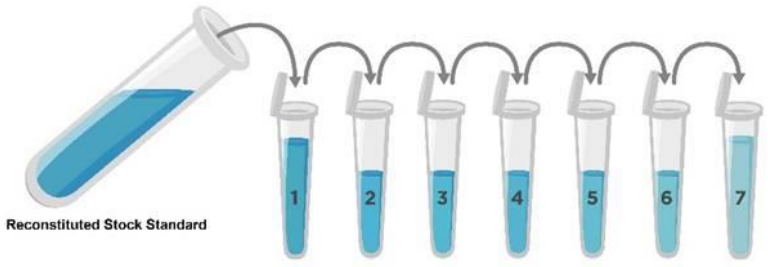
8.1.3.4 Prepare **Standard #2** by adding 300 µL of **Standard #1** (Tube #1) to Tube #2. Mix gently and thoroughly.

8.1.3.5 Prepare **Standard #3** by adding 300 µL of **Standard #2** from Tube #2 to Tube #3. Mix gently and thoroughly.

8.1.3.6 Prepare further serial dilutions through Tube #7. Reference the table below as a guide for serial dilution scheme.

8.1.3.7 Tube #8 is a blank standard (only **Standard Diluent**), which should be included with every experiment.

Standard Number (Tube)	Standard To Dilute	Volume Standard to Dilute (µL)	Volume Standard Diluent Buffer (µL)	Total Volume (µL)	Final Concentration
1	10,000 pg/mL	200	800	1,000	2,000 pg/mL
2	2,000 pg/mL	300	600	900	666.6 pg/mL
3	666.6 pg/mL	300	600	900	222.2 pg/mL
4	222.2 pg/mL	300	600	900	74.07 pg/mL
5	74.07 pg/mL	300	600	900	24.69 pg/mL
6	24.69 pg/mL	300	600	900	8.23 pg/mL
7	8.23 pg/mL	300	600	900	2.74 pg/mL
8	NA	0	600	600	0.0 (Blank)



8.2 1X Biotinylated NPPB Detector Antibody

8.2.1 Prepare the **1X Biotinylated NPPB Detector Antibody** immediately prior to use by diluting the **100X Biotinylated NPPB Detector Antibody** 1:100 with **Detector Antibody Diluent**.

8.2.2 For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of **100X Biotinylated NPPB Detector Antibody** to 990 µL **Detector Antibody Diluent**.

8.2.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

8.3 1X Avidin-HRP Conjugate

8.3.1 Prepare the **1X Avidin-HRP Conjugate** immediately prior to use by diluting the **100X Avidin-HRP Conjugate** 1:100 with **Conjugate Diluent**.

8.3.2 For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of **100X Avidin-HRP Conjugate** to 990 µL **Conjugate Diluent**.

8.3.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

8.4 1X Luminol Substrate

8.4.1 Prepare the **1X Luminol Substrate** immediately prior to use by diluting the **100X Luminol Substrate** 1:100 with **Substrate Diluent**.

8.4.2 For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of **100X Luminol Substrate** to 990 µL **Substrate Diluent**.

8.4.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

8.5 1X Wash Buffer

8.5.1 If crystals have formed in the 30X **Wash Buffer** concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.

8.5.2 Add the entire 20 mL contents of the 30X **Wash Buffer** bottle to 580 mL of ultra-pure water to a clean > 1,000 mL bottle or other vessel.

8.5.3 Seal and mix gently by inversion. Avoid foaming or bubbles.

8.5.4 Store the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

8.6 Microplate Preparation

- Micro-plates are provided ready to use and do not require rinsing or blocking.
- Unused well strips should be returned to the original packaging, sealed and stored at 4°C.
- Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

9. Sample Preparation

9.1 Sample Preparation and Storage

• Store samples to be assayed at 2-8°C for 24 hours prior being assayed.

• For long term storage, aliquot and freeze samples at -20°C.Avoid repeated freeze-thaw cycles.

• Samples not indicated in the manual must be tested to determine if the kit is valid.

• Prepare samples as follows:

• **Serum** - Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1,000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

• **Plasma** - Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

• **Tissue Homogenates** – Rinse 100 mg of tissue with 1X PBS, then homogenize in 1 mL of 1X PBS and store overnight at -20°C. Perform two freeze-thaw cycles to break the cell membranes, then centrifuge the homogenate for 5 minutes at 5,000 x g, 2-8°C. Remove the supernatant and assay immediately. Alternatively, aliquot and store samples at -20°C or -80°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

• **Cell Lysates -** Adherent cells should be detached with trypsin and then collected by centrifugation (suspension cells can be collected by centrifugation directly). Wash cells three times in cold PBS. Resuspend cells in PBS (1×) and ultrasonicate the cells 4 times (or freeze cells at ≤ -20°C. Thaw cells with gentle mixing. Repeat the freeze/thaw cycle 3 times.) Centrifuge at 1,500 x g for 10 minutes at 2 - 8°C to remove cellular debris.

• **Cell culture supernatants and other biological fluids** – Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

10. Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

• Optimal results for intra- and inter-assay reproducibility will be obtained when performing incubation steps at 37°C as indicated below.

10.1 Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.

10.2 Add 100 µL of serially titrated standards, diluted samples or blank into wells of the **Anti-NPPB Microplate**. At least two replicates of each standard, sample or blank is recommended.

10.3 Cover the plate with the well plate lid and incubate at 37°C for 2 hours.

10.4 Remove the plate lid and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.5 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.6 Add 100 µL of prepared **1X Biotinylated NPPB Detector Antibody** to each well.

10.7 Cover with the well-plate lid and incubate at 37°C for 60 minutes.

10.8 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.9 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.10 Wash plate 3 times with **1X Wash Buffer** as follows:

10.10.1 Add 300 µL of **1X Wash Buffer** to each assay well.

10.10.2 Incubate for 1-2 minute.

10.10.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.10.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.10.5 Repeat steps 10.10.1 through 10.10.4 **two** more times.

10.11 Add 100 µL of prepared **1X Avidin-HRP Conjugate** into each well and incubate at 37°C for 30 minutes.

10.12 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.13 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.14 Wash plate **5 times** with **1X Wash Buffer** as in Step 10.10.

10.15 Add 100 µL of **1X Luminol Substrate** to each well. Cover the plate with a well seal and incubate at 37°C for 5-10 minutes **in the dark**.

10.16 Read the RLU (relative light units) with a standard luminometer or photo-multiplier tube equipped instrument.

11. Calculation of Results

For analysis of the assay results, calculate the **Relative Light Units (RLU)** for each test or standard well as follows

(Relative Light Units) = (Mean Sample Well Light Unit Emission) – (Mean Blank Well Light Unit Emission)

The standard curve is generated by plotting the mean replicate **RLU** of each standard serial dilution point vs. the respective standard concentration. The **NPPB** concentration contained in the samples can be interpolated by using linear regression of each mean sample **RLU** against the standard curve. This is best achieved using curve fitting software.

Note: If the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

12. Typical Expected Data

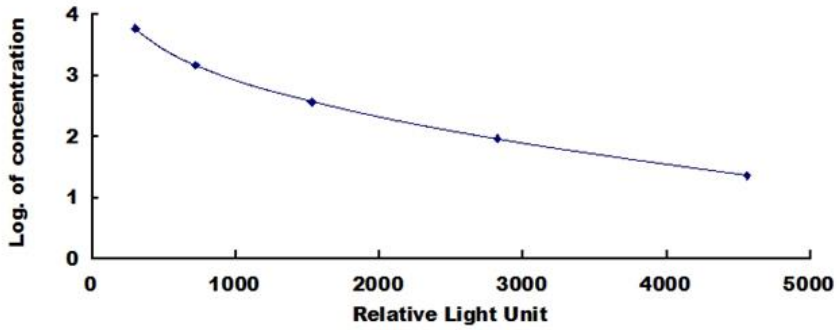
12.1 Reproducibility

Intra-assay Precision: 3 samples with known low, middle and high levels NPPB were tested with 20 replicates on one plate, respectively. Inter-assay Precision: 3 samples with known low, middle and high level NPPB were tested on 3 different plates, 8 replicates in each plate.

Sample	Intra-Assay			Inter-Assay		
	1	2	3	1	2	3
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (ng/ml)						
SD						
CV (%)						

12.2 Typical standard curve

This standard curve is for demonstration purposes only. An assay specific standard curve should be performed with each assay.



12.3 Linearity

Kit linearity evaluated by testing serially diluted samples containing known concentrations of NPPB. Results are expressed as the percentage of the expected concentration measurement.

Sample Type	Dilution Level			
	1:2	1:4	1:8	1:16
Serum (n=5)				
EDTA Plasma (n=5)				
Heparin Plasma (n=5)				

12.4 Recovery

The following matrices were spiked with known concentration of NPPB. Recovery is expressed as the percentage of the expected concentration measurement.

Sample Type	Recovery Range (%)	Average (%)
Serum(n=5)		
EDTA Plasma(n=5)		
Heparin Plasma(n=5)		

13. Technical Resources

Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

USA

Aviva Systems Biology, Corp.
5754 Pacific Center Blvd, Suite 201
San Diego, CA 92121

Phone: 858-552-6979
Toll Free: 888-880-0001
Fax: 858-552-6975

Technical support: techsupport@avivasysbio.com

China

Beijing AVIVA Systems Biology
6th Floor, B Building, Kaichi Tower
#A-2 Jinfu Road.
Daxing Industrial Development Zone
Beijing, 102600, CHINA

Phone: (86)10-60214720
Fax: (86)10-60214722
E-mail: support@avivasysbio.com.cn

中国地址：北京大兴工业开发区金辅路甲 2 号凯驰大厦 B 座 6 层 (102600)
电话: 010-60214720/21
传真: 010-60214722

产品售前咨询及销售: sales@avivasysbio.com.cn
售后及技术支持: support@avivasysbio.com.cn