ab108815 – BNP 32 Rat ELISA Kit

Instructions for Use

For the quantitative measurement of Rat BNP 32 in plasma, serum, tissue extract, and cell culture supernatants.

This product is for research use only and is not intended for diagnostic use.

Version 11a Last Updated 13 September 2023

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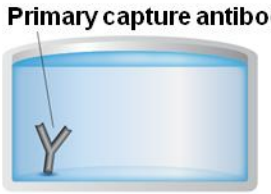
1. BACKGROUND

Abcam's BNP 32 Rat *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of BNP 32 in plasma, serum, tissue extract, and cell culture supernatants.

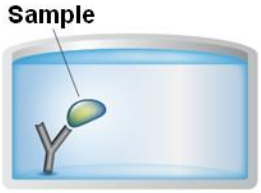
A BNP 32 specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently a BNP 32 specific biotinylated detection antibody is added and then followed by washing with wash buffer. Streptavidin-Peroxidase Conjugate is added and unbound conjugates are washed away with wash buffer. TMB is then used to visualize Streptavidin-Peroxidase enzymatic reaction. TMB is catalyzed by Streptavidin-Peroxidase to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the amount of BNP 32 captured in plate.

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. A high level of plasma BNP may have a strong, independent association with increased mortality rates in patients with primary pulmonary hypertension, congestive heart failure and/or after acute myocardial infarction.

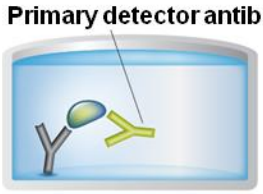
2. ASSAY SUMMARY



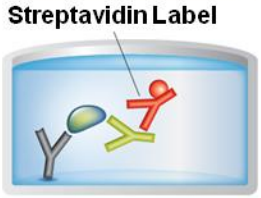
Prepare all reagents, samples and standards as instructed.



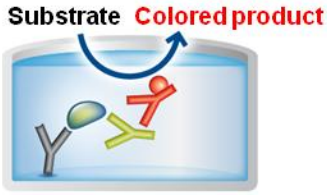
Add standard or sample to each well used. Incubate at room temperature.



Wash and add prepared biotin antibody to each well. Incubate at room temperature.



Wash and add prepared Streptavidin-Peroxidase Conjugate. Incubate at room temperature.



Add Chromogen Substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 4°C immediately upon receipt, apart from the SP Conjugate & Biotinylated Antibody, which should be stored at -20°C.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
BNP 32 Microplate (12 x 8 well strips)	96 wells	4°C
BNP 32 Standard	1 vial	4°C
10X Diluent N Concentrate	30 mL	4°C
Biotinylated Rat BNP 32 Antibody	1 vial	-20°C
100X Streptavidin-Peroxidase Conjugate (SP Conjugate)	80 µL	-20°C
Chromogen Substrate	7 mL	4°C
Stop Solution	11 mL	4°C
20X Wash Buffer Concentrate	2 x 30 mL	4°C
Sealing Tapes	3	N/A

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 1 Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 1 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- 8 tubes to prepare standard or sample dilutions.

7. LIMITATIONS

- Do not mix or substitute reagents or materials from other kit lots or vendors.

8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

Equilibrate all reagents to room temperature (18-25°C) prior to use. Prepare fresh reagents immediately prior to use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved, and ensure to rinse the bottles thoroughly to extract any precipitates left in the bottle.

9.1 **1X Diluent N**

Dilute the 10X Diluent N Concentrate 1:10 with reagent grade water. Mix gently and thoroughly. *Store for up to 1 month at 4°C.*

9.2 **1X Wash Buffer**

Dilute the 20X Wash Buffer Concentrate 1:20 with reagent grade water. Mix gently and thoroughly.

9.3 **1X Biotinylated BNP 32 Detector Antibody**

9.3.1 The stock Biotinylated BNP 32 Antibody must be diluted with 1X Diluent N according to the label concentration to prepare 1X Biotinylated BNP 32 Antibody for use in the assay procedure. Observe the label for the "X" concentration on the vial of Biotinylated BNP 32 Antibody.

9.3.2 Calculate the necessary amount of 1X Diluent N to dilute the Biotinylated BNP 32 Antibody to prepare a 1X Biotinylated BNP 32 Antibody solution for use in the assay procedure according to how many wells you wish to use and the following calculation:

Number of Wells Strips	Number of Wells	(V_T) Total Volume of 1X Biotinylated Antibody (µL)
4	32	1,760
6	48	2,640
8	64	3,520
10	80	4,400
12	96	5,280

Any remaining solution should be frozen at -20°C.

Where:

C_S = Starting concentration (X) of stock Biotinylated BNP 32 Antibody (variable)

C_F = Final concentration (always = 1X) of 1X Biotinylated BNP 32 Antibody solution for the assay procedure

V_T = Total required volume of 1X Biotinylated BNP 32 Antibody solution for the assay procedure

V_A = Total volume of (X) stock Biotinylated BNP 32 Antibody

V_D = Total volume of 1X Diluent N required to dilute (X) stock Biotinylated BNP 32 Antibody to prepare 1X Biotinylated BNP 32 solution for assay procedures

Calculate the volume of (X) stock Biotinylated Antibody required for the given number of desired wells:

$$(C_F / C_S) \times V_T = V_A$$

Calculate the final volume of 1X Diluent N required to prepare the 1X Biotinylated BNP 32 Antibody:

$$V_T - V_A = V_D$$

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your antibody vial for the actual concentration of antibody provided.

C_S = 50X Biotinylated BNP 32 Antibody stock

C_F = 1X Biotinylated BNP 32 Antibody solution for use in the assay procedure

V_T = 3,520 µL (8 well strips or 64 wells)

$$(1X/50X) \times 3{,}520\ \mu L = 70.4\ \mu L$$

$$3{,}520\ \mu L - 70.4\ \mu L = 3{,}449.6\ \mu L$$

V_A = 70.4 µL total volume of (X) stock Biotinylated BNP 32 Antibody required

V_D = 3,449.6 µL total volume of 1X Diluent N required to dilute the 50X stock Biotinylated Antibody to prepare 1X Biotinylated BNP 32 Antibody solution for assay procedures

9.3.3 First spin the Biotinylated BNP 32 Antibody vial to collect the contents at the bottom.

9.3.4 Add calculated amount V_A of stock Biotinylated BNP 32 Antibody to the calculated amount V_D of 1X Diluent N. Mix gently and thoroughly.

9.4 **1X SP Conjugate**

Spin down the 100X Streptavidin-Peroxidase Conjugate (SP Conjugate) briefly and dilute the desired amount of the conjugate 1:100 with 1X Diluent N.

Any remaining solution should be frozen at -20°C.

10. STANDARD PREPARATIONS

- **Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.**
- **Any remaining standard should be stored at -20°C after reconstitution and used within 30 days.**
- **This procedure prepares sufficient standard dilutions for duplicate wells.**

10.1 Reconstitution of the BNP 32 Standard vial to prepare the 2 ng/mL BNP 32 **Standard #1**.

10.1.1 First consult the BNP 32 Standard vial to determine the mass of protein in the vial.

10.1.2 Calculate the appropriate volume of 1X Diluent N to add when resuspending the BNP 32 Standard vial to produce a 2 ng/mL BNP 32 **Standard #1** by using the following equation:

C_S = Starting mass of BNP 32 Standard (see vial label) (ng)
C_F = The 2 ng/mL BNP 32 **Standard #1** final required concentration
V_D = Required volume of 1X Diluent N for reconstitution (µL)

Calculate total required volume 1X Diluent N for resuspension:

$$(C_S / C_F) \times 1{,}000 = V_D$$

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.

C_S = 0.8 ng of BNP 32 Standard in vial
C_F = 2 ng/mL BNP 32 **Standard #1** final concentration
V_D = Required volume of 1X Diluent N for reconstitution

$$(0.8 \text{ ng} / 2 \text{ ng/mL}) \times 1{,}000 = 400 \text{ µL}$$

10.1.3 First briefly spin the BNP 32 Standard Vial to collect the contents on the bottom of the tube.

10.1.4 Reconstitute the BNP 32 Standard vial by adding the appropriate calculated amount V_D of 1X Diluent N to the vial to generate the 2 ng/mL BNP 32 **Standard #1**. Mix gently and thoroughly.

10.2 Allow the reconstituted 2 ng/mL BNP 32 **Standard #1** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions

10.3 Label seven tubes #2 – 8.

10.4 Add 120 µL of 1X Diluent N to tube #2 – 8.

10.5 To prepare **Standard #2**, add 120 µL of the **Standard #1** into tube #2 and mix gently.

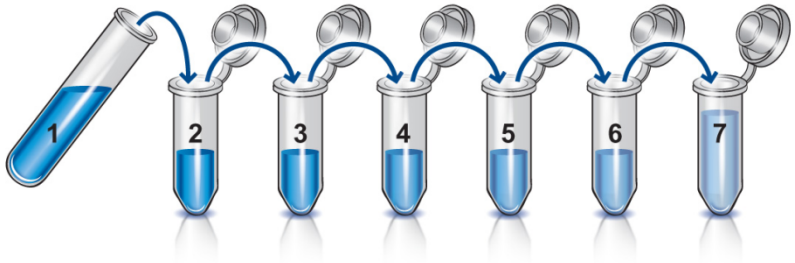
10.6 To prepare **Standard #3**, add 120 µL of the **Standard #2** into tube #3 and mix gently.

10.7 Using the table below as a guide, prepare subsequent serial dilutions.

10.8 1X Diluent N serves as the zero standard, 0 ng/mL (tube #8).

Standard Dilution Preparation Table

Standard #	Volume to Dilute (µL)	Volume Diluent N (µL)	Total Volume (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	Step 10.1				2.000
2	120	120	240	2.000	1.000
3	120	120	240	1.000	0.500
4	120	120	240	0.500	0.250
5	120	120	240	0.250	0.125
6	120	120	240	0.125	0.063
7	120	120	240	0.063	0.031
8	-	120	120	-	0



11. SAMPLE PREPARATION

11.1 Plasma

Collect plasma using a final concentration of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3,000 x *g* for 10 minutes and assay undiluted plasma for medium and high level of BNP 32. Samples can be diluted with Diluent N if required; the user should determine the optimal dilution factor depending on application needs. Undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.2 Cell Culture Supernatants

Centrifuge cell culture media at 1500rpm for 10 minutes at 4°C to remove debris, then collect supernatant. Samples can be diluted with Diluent N if required; the user should determine the optimal dilution factor depending on application needs. Undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

11.3 Serum

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3,000 x *g* for 10 minutes, then remove serum. Perform the assay with undiluted samples for medium and high level of BNP 32. Samples can be diluted with Diluent N if required; the user should determine the optimal dilution factor depending on application needs. Undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.4 Tissue

Extract tissue samples with 0.1 M phosphate-buffered saline (pH 7.4) containing 1% Triton X-100 and centrifuge at 14,000 x g for 20 min. Collect the supernatant and measure the protein concentration. Perform the assay with undiluted samples for medium and high levels of BNP 32. Samples can be diluted with Diluent N if required; the user should

determine the optimal dilution factor depending on application needs. Undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well plate strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature (18 - 25°C) prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1 Prepare all reagents, working standards and samples as instructed. Equilibrate reagents to room temperature before use. The assay is performed at room temperature (18-25°C).

13.2 Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.

13.3 Add 50 µL of BNP 32 Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.

13.4 Wash five times with 200 µL of 1X Wash Buffer manually. Invert the plate each time and decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µL of 1X Wash Buffer and then invert the plate, decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid.

13.5 Add 50 µL of 1X Biotinylated BNP 32 Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for two hours.

13.6 Wash microplate as described above.

13.7 Add 50 µL of 1X SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and

incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.

13.8 Wash microplate as described above.

13.9 Add 50 µL of Chromogen Substrate to each well, Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate in ambient light for 25 minutes until the optimal blue color density develops.

13.10 Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.

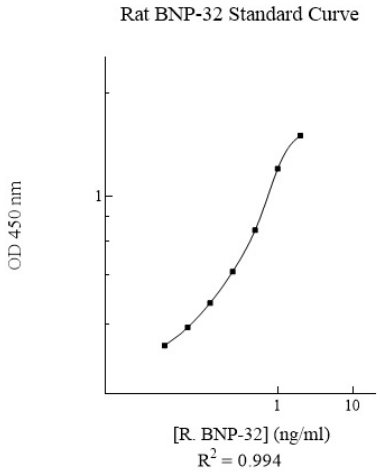
13.11 Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

14. CALCULATIONS

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 log-log or four-parameter logistic curve-fit. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



TYPICAL SAMPLE VALUES

SENSITIVITY –

The minimum detectable dose of BNP 32 is typically ~ 18 pg/mL.

RECOVERY –

Standard Added Value: 0.031 – 2 ng/mL

Recovery %: 82 – 114

Average Recovery %: 103

LINEARITY OF DILUTION –

Plasma Dilution	Average % Expected Value
1X	115
2X	105
4X	89

Serum Dilution	Average % Expected Value
1X	89
2X	98
4X	112

PRECISION –

	Intra-Assay	Inter-Assay
% CV	4.9	10.7

16. ASSAY SPECIFICITY

Species	% Cross Reactivity
Canine	40
Bovine	None
Equine	20
Monkey	100
Mouse	10
Swine	100
Rabbit	None
Human	30

17. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Improper standard dilution	Confirm dilutions made correctly
	Standard improperly reconstituted (if applicable)	Briefly spin vial before opening; thoroughly resuspend powder (if applicable)
	Standard degraded	Store sample as recommended
	Curve doesn't fit scale	Try plotting using different scale
Low signal	Incubation time too short	Try overnight incubation at 4°C
	Target present below detection limits of assay	Decrease dilution factor; concentrate samples
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes and ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (eg. minimize freeze/thaws cycles)

Problem	Cause	Solution
High background/ Low sensitivity	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding STOP solution	Read plate immediately after adding STOP solution
	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan