BNP32 ELISA

Cat. No: KBI5002
Ver1.0

Immunoassay for quantitative estimation of BNP32 in human serum, plasma and tissue extracts.

KRISHGEN BioSystems | Unit Nos#318/319, Shah & Nahar, Off Dr E Moses Road, Worli, Mumbai 400 018.
Tel: (022) 49198700 Email: sales@krishgen.com

KRISHGEN BioSystems

BNP32 ELISA

Introduction:
BNP32 (Nesiritide/Natrecor) is an endogenous peptide secreted from cardiac ventricles in response to volume increase and pressure overload that acts as an agonist at atrial natriuretic peptide (ANP) receptor A (NRP1). In vitro: BNP 1-32 (10(-6) mol/L) activats cGMP. BNP significantly (P<0.01) inhibited de novo collagen synthesis as assessed by [3H]proline incorporation, whereas zymographic MMP-2 (gelatinase) abundance was significantly (P<0.05) stimulated by BNP between 10(-7) and 10(-6) mol/L. Decreases de novo collagen synthesis and increases MMP gene expression in vitro. In vivo: inhibits the sympathetic and renin-angiotensin-aldosterone systems.

Intended Use:
The BNP32 ELISA is used for estimation of BNP32 in serum, plasma, or tissue extracts in pharmacokinetics, peptide delivery study and other purposes.

Principle:
The BNP32 ELISA is a sandwich immunoassay for the determination of BNP32. The antibodies coated on a 96-well plate capture the standard or sample. BNP32 antiserum is subsequently bound to this complex. Biotin is then added followed by SA-HRP (streptavidin-conjugated horseradish peroxidase), which produces a soluble colored product after a substrate is added. The enzyme reaction is stopped by dispensing an acidic solution (H2SO4) into the wells after 10 min at room temperature turning the solution from blue to yellow. The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific BNP32 bound.

Materials Provided:

1. Microtitre coated plate (96 wells) – 1 no
2. Biotin Concentrate (lyophilized) – 1 vial
3. BNP32 Standard (lyophilized) - 1 vial
4. Standard Diluent - 1 bottle
5. Assay Buffer (20X), 50ml -1 bottle
6. BNP32 Antiserum (lyophilized) - 1 bottle
7. Streptavidin-HRP (200X), 100 ul - 1 vial
8. TMB Substrate, 11ml – 1 bottle
9. Stop Solution, 15ml – 1 bottle
10. Instruction Manual

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50µl to 1000µl.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Timer.
6. Absorbent paper
7. Test tubes, pipettes and various other standard laboratory items

8. Extraction Kit (optional, recommended) Catalog No#KBI9010ext

Handling/Storage:

1. Reconstitute or dilute only the specific reagents mentioned in the reagent preparation section, when ready to run the assay.
2. After receiving the kit, store the lyophilized components at a constant -20°C for up to one year from the kit's expiration date. The remaining components should be stored in the refrigerator (2- 4°C) up to the kit's expiration date.
3. Do not use kit components after the expiration date.
4. Do not repeatedly freeze/thaw the reagents as loss of activity may result.
5. Before using, bring all components to Room Temperature (18-25ºC). Upon assay completion return all components to appropriate storage conditions.
6. ELISA plate pouches contain dessicant. Keep the plates sealed in the pouch with dessicant in the refrigerator when not in use.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. Handle Stop Solution carefully. Obtain medical attention in case of accidental ingestion of kit components.
3. Avoid assay of samples containing Sodium azide as it is hazardous.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. 1X Assay Buffer: To make 1X Assay Buffer, add 50 ml of 20X Assay Buffer to 950ml of DI water. This is the working solution.
2. BNP32 Antiserum: Add 5 ml of Assay Buffer and vortex.
3. Biotin Concentrate: Add 5 ml of Assay Buffer to the vial of lyophilized biotinylated peptide and vortex.
4. Standard: Prepare the standards using the schematic provided below using the Standard Diluent provided in the Kit.

Suggested Preparation of Standards		
	ng/ml	**Range: 1 to 1000 ng/ml**
Stock	**1000**	
S5	1000	Add 200 ul of stock
S4	100	Add 20 ul stock + 180 ul Standard Diluent
S3	10	Add 20 ul S4 + 180 Standard Diluent
S2	1	Add 20 ul S3 + 180 Standard Diluent
S1	0	500 ul Standard Diluent

Specimen Collection and Preparation:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

Serum: Use a serum separator tube and allow clotting for 30 minutes, then centrifuge for 10 minutes at 1000 x *g*. Remove serum layer and assay immediately or store serum samples at < -20°C. Avoid repeated freeze/thaw cycles.

Dilute the serum 1:50 in Sample Diluent. For example: add 5 ul of serum sample to 245 ul of 1X Sample Diluent. If not assayed immediately, diluted samples should be stored at -20°C or below.

Sample Extraction. Factors present in serum may bind to the antiserum complex. The effects can vary from negligible to complete obliteration of signal. Sample extraction may, therefore, be required prior to using the kit. It is recommended to use the KRISHGEN Extraction Kit (optional, recommended) Catalog No#KBI9010ext. The kit may work for some tissue culture samples without the extraction. See "Protocol for Sample Extraction" in the data sheet / kit insert / IFU of Catalog No.#KBI9010ext. The kit may still be used without extraction but this may cause unexpected results due to the possible binding between serum proteins and kit components.

Procedural Notes:

1. Read all the instructions thoroughly before performing the test.
2. Allow all reagents to reach Room Temperature before beginning and reconstitute or dilute the required reagents.
3. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess unreacted reagents is essential.
4. All Standards, Controls and Samples should be assayed at least in duplicates.
5. The assay has been optimized to be used with the protocol mentioned. Any deviation from the same may invalidate the results.

Assay Procedure:

1. Bring all reagents to Room Temperature prior to use. It is strongly recommended that all **Controls and Samples** should be run in duplicates or triplicates.
2. Pipette 50 ul **Standard** or **Sample** (in Assay Buffer) and 25 ul **BNP32 Antiserum** (in Assay Buffer) into each respective microwell except the blank microwells.
3. Add 75 ul **Assay Buffer** to the blank microwells.
4. Pipette 25 ul of the reconstituted **Biotin Concentrate** into each microwell.
5. Aspirate and wash plate 5 times with **Assay Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly or alternatively a microtiter plate or strip washer may be used.
6. Add 100 ul of freshly prepared **Streptavidin-HRP**. *Tap or centrifuge the SA-HRP vial to collect all liquid contents on the bottom of the vial. Dilute 1/200 in Assay Buffer (60 ul / 12 ml) and vortex. Add 100 ul to all wells, including the blanks.*
7. Incubate at Room Temperature for 60 minutes.
8. Wash the plate as per the Procedure given in step 5 above.
9. Add 100 ul of **TMB Substrate** into each microwell of the plate.

10. Cover the plate with a para film and incubate at Room Temperature under dark for 30-60 minutes.

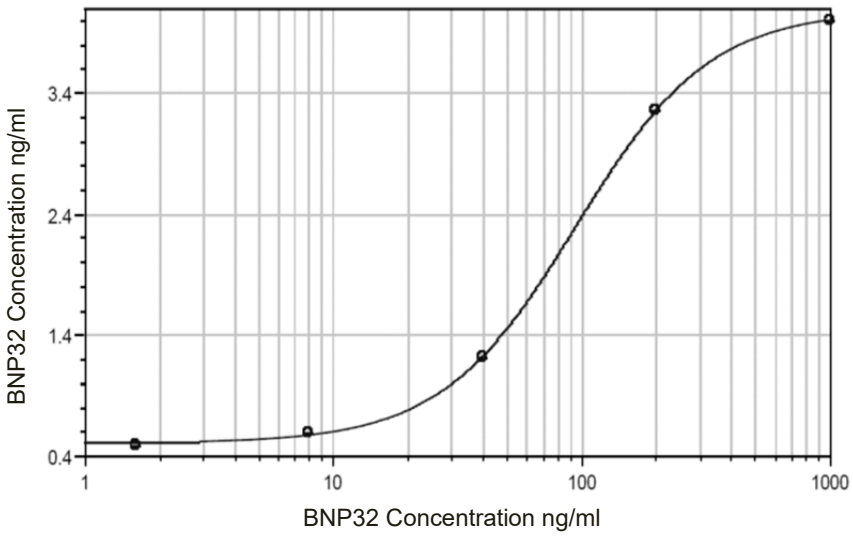
11. Add 100 ul of **Stop Solution** to each microwell.

12. Measure the optical density of the wells on a plate reader at 450 nm within 10 minutes.

Interpretation of the Results:

Plot data and calculate results. We recommend that you use curve fitting software for your data analysis. Plate readers often include such software packages. This is, however, not essential and you may opt to plot manually on semi-log paper. You can also use a spreadsheet program.

We recommend log / lin processing for best results. The standard curve is established by plotting the mean OD-values of the calibrators on the ordinate, y-axis, (lin. scale) versus their respective BNP32 concentrations on the abscissa, x-axis, (log. scale). BNP32 concentrations of the unknown samples are directly read off in ng/ml against the respective OD values.



Precautions:

1. Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

2. Substrate is light and heat sensitive hence do not expose it to direct sunlight while pipetting or incubating.

3. Samples and kit reagents after use should be disposed off observing appropriate regulations.

4. If necessary it is recommended that the results should be confirmed by an alternative method.

5. Do not dilute or adulterate test reagents or use samples not called for in the test procedure.

Performance Characteristics:

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay and the dilutional linearity assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

1. Specificity:
The antibodies in this kit are specific for BNP32 peptide. The standard used is a synthetic peptide for BNP32.

Peptide Sequence: H - Ser - Pro - Lys - Met - Val - Gln - Gly - Ser - Gly - Cys - Phe - Gly - Arg - Lys - Met - Asp - Arg - Ile - Ser - Ser - Ser - Ser - Gly - Leu - Gly - Cys - Lys - Val - Leu - Arg - Arg - His - OH (Disulfide bridge: 10 - 26)

2. Cross Reactivity:

BNP32:	100%
Pro BNP (1-76):	0%

3. Precision:
The kit's IC50, or the shape of the standard curve, may exhibit some variation but this will not affect the kit's accuracy in the measuring range. The kit accurately measures sample peptides if the following conditions are met.
i) Both samples and standards must be measured in the same diluent and under the same conditions.
ii) The kit's antibodies must not cross-react appreciably with other factors present in the sample. The user may wish to test the cross- reactivity with other peptides.
iii) The sample peptides must be identical to the standard. Ideally the synthetic standard mimics the natural peptide perfectly. Sometimes, however, natural peptides exist as families of species related by a common or similar sequence. In addition, natural peptides may be enzymatically or spontaneously modified, may exist in complexes, and may assume alternative structural forms. In these cases the kit might not measure the exact concentration of a particular natural peptide species, but, it may still be used for relative average measurements.
iv) Sample extraction. Factors present in serum can bind to kit components. The effects can vary from negligible to complete obliteration of signal. Sample extraction may, therefore, be required prior to using the kit.

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

THANK YOU FOR USING KRISHGEN PRODUCT!