AssayMax Rat BNP-32 (rBNP-32) ELISA Kit

Catalog Number ERB1201-1

Introduction

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).

A high level of plasma BNP may have a strong, independent association with increased mortality rates in patients with primary pulmonary hypertension (PPH) (2), congestive heart failure and/or after acute myocardial infarction (3, 4).

Principal of the Assay

The AssayMax rBNP-32 ELISA kit is designed for detection of rat BNP-32 in plasma, serum, tissue extract and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique which measures rBNP-32 in 4 hours. A polyclonal antibody specific for rBNP-32 has been pre-coated onto a microplate. The rBNP-32 in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for rBNP-32, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **rBNP-32 Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against rBNP-32.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes which can be cut to fit the format of the individual assay.
- **rBNP-32 Standard:** Rat BNP-32 in a buffered protein base (2 ng, lyophilized).
- **Biotinylated rBNP-32 Antibody (70x):** A 70-fold biotinylated polyclonal antibody against rBNP-32 (120 µl).
- **Streptavidin-Peroxidase Conjugate (S. P. Conjugate):** A 100-fold concentrate (120 µl).

- **EIA Diluent Concentrate (10x):** A 10-fold buffered protein base (20 ml).
- **Wash Buffer Concentrate (10x)**: A 10-fold concentrated buffered surfactant (2x30 ml).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydroxychloric acid (12 ml) to stop the chromogen substrate reaction.

Storage Condition

- Store unopened kit at 2 - 8^0C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2 - 8^0C. Store reconstituted standard and S. P. Conjugate concentrate at -20^0C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

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

Sample Collection and Storage

- **Plasma:** Collect plasma using a final concentration of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2,000x g for 10 minutes and assay undiluted plasma for medium and high level of BNP-32. Samples can be stored at -20^0C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

  For low level of BNP-32, please use the extraction protocol as follows:

  <u>BNP-32 extraction protocol</u>

  Buffer A: 1% trifluoroacetic acid (TFA, HPLC Grade) in H_2O
  Buffer B: 60% acetonitrile (HPLC Grade) in 1% TFA

  1. Acidify the sample with equal amount of Buffer A (1 ml sample : 1 ml Buffer A). Mix and centrifuge samples at 6,000 x g for 20 minutes at 4°C.
  2. Pack an extraction column using 200 mg of C18 resin. Pre-equilibrate the column with 1 ml of Buffer B once and then with 3 ml of Buffer A three times.
  3. Load the acidified plasma solution onto the pre-treated C18 column.
  4. Slowly wash the column with 3 ml of Buffer A twice.
  5. Elute the peptide slowly with 3 ml of Buffer B once and collect the eluant.
  6. Evaporate and dry the eluant in a freeze dryer or use a suitable substitute method.
  7. Keep the dried extract at -20°C and perform the assay as early as possible. Reconstitute the dried extract with 200 µl of EIA Diluent before the assay. Check sample pH with pH papers. If sample pH is below 6.5, neutralize the sample with 20 µl of 1M NaH_2PO_4. If the peptide value exceeds or does not fall in the range of detection, dilute or concentrate the sample accordingly.

- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes. Remove serum and perform the assay for medium and high level of BNP-32. Samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

  For low level of BNP-32, please use the extraction protocol as above.

- **Tissue:** Extract tissue samples with 0.1 M phosphate-buffered saline (pH7.4) containing 1% Triton X-100 and centrifuge at 14,000 x g for 20 min. Collect the supernatant and measure the protein concentration. Dilute the tissue extract 1:20 into EIA diluent and assay. Freeze the remaining extract at -20^{0}C or below.

- **Cell Culture Supernatants:** Centrifuge cell culture media at 2,000x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20^{0}C or below. Avoid repeated freeze-thaw cycles. Dilute samples 1:4 with EIA diluent and assay.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **EIA Diluent Concentrate (10x):** Dilute the EIA Diluent Concentrate 1:10 with reagent grade water.
- **rBNP-32 Standard:** Reconstitute the 2 ng of rat BNP-32 Standard with 1 ml of EIA Diluent to generate a stock solution of 2 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the Standard (2 ng/ml) twofold with EIA Diluent to generate 1, 0.5, 0.25, 0.125, 0.0625, 0.03125 ng/ml. EIA Diluent serves as the zero standard (0 ng/ml).

Standard Point	Dilution	[rBNP-32] (ng/ml)
P1	1 part Standard (2 ng/ml)	2.000
P2	1 part P1 + 1 part EIA Diluent	1.000
P3	1 part P2 + 1 part EIA Diluent	0.500
P4	1 part P3 + 1 part EIA Diluent	0.250
P5	1 part P4 + 1 part EIA Diluent	0.125
P6	1 part P5 + 1 part EIA Diluent	0.063
P7	1 part P6 + 1 part EIA Diluent	0.031
P8	EIA Diluent	0.000

- **Biotinylated rBNP-32 Antibody (70x):** Dilute the antibody 1:70 with EIA Diluent. Spin down the Biotinylated Antibody briefly and only dilute the desired amount of the antibody.
- **Wash Buffer Concentrate (10x):** Dilute the Wash Buffer Concentrate 1:10 with reagent grade water.
- **S. P. Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent.

.

Assay Procedure

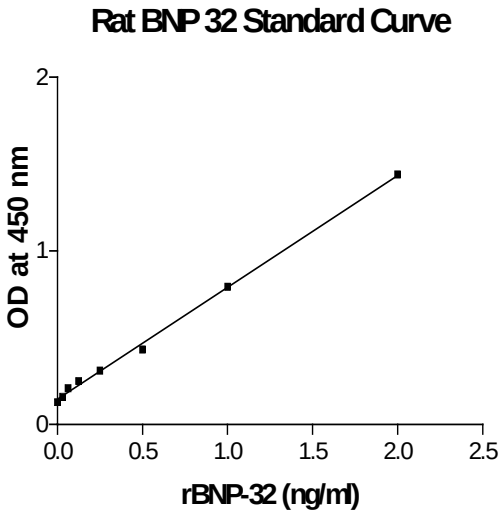
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30^{0}C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Standard or sample per well, and cover wells and incubate for 2 hours. Start the timer after the last sample addition.
- Wash four times with 200 µl of Wash Buffer. Invert the plate and decant the contents, and blot it on absorbent paper towel to complete remove liquid at each step.
- Add 50 µl of Biotinylated rBNP-32 Antibody to each well and incubate for 60 minutes.
- Wash four times with 200 µl of Wash Buffer.
- 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.
- Wash four times with 200 µl of Wash Buffer.
- Add 50 µl of Chromogen Substrate per well and incubate for approximately 10 minutes or till the optimal color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm immediately.

Data Analysis

- 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 and draw a best fit curve through the points on the graph. Plotting the 4 parameters or linear graph may linearize the data and the best-fit line can be determined by regression analysis of the linear portion of the curve.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

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-32 is typically 10 pg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.8 % and 7.9% respectively.

References

1. Wiedemann K, Jahn H, Kellner M *Exp Clin Endocrinol Diabetes* 2000;108(1):5-13
2. Nagaya N. *et al. Circulation* 2000 Aug 22;102(8):865-70
3. Cheng V *et al. J Am Coll Cardiol* 2001 Feb;37(2):386-91
4. Bettencourt P. *et al. Clin Cardiol* 2000 Dec;23(12):921-7

Revision 5.2.3