



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

Catalog No. 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, and tissue extract and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures rBNP-32 in less than 5 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 that can be cut to fit the format of the individual assay.
- **rBNP-32 Standard:** Rat BNP-32 in a buffered protein base (4 ng, lyophilized).
- **Biotinylated rBNP-32 Antibody (100x):** A 100-fold biotinylated polyclonal antibody against rBNP-32 (80 µl).
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (90 µl).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).

- **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Store kit at 2-8⁰C or -20⁰C upon arrival up to the expiration date.
- Opened MIX Diluent may be stored for up to 1 month at 2-8⁰C. Store reconstituted reagents at -20⁰C 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, 200-1000 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 2000x g for 10 minutes and assay undiluted plasma for medium and high level of BNP-32. Samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and perform the assay for medium and high level of BNP-32. Samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Tissue:** Extract tissue samples with 0.1 M phosphate-buffered saline (pH7.4) containing 1% Triton X-100 and centrifuge at 14000 x g for 20 min. Collect the supernatant and measure the protein concentration. Dilute the tissue extract 1:20 into MIX diluent and assay. Freeze the remaining extract at -20⁰C or below.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20⁰C or below. Avoid repeated freeze-thaw cycles. Dilute samples 1:4 with MIX 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.
- **MIX Diluent Concentrate (10x):** Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2 - 8⁰C.
- **rBNP-32 Standard:** Reconstitute the 4 ng of rat BNP-32 Standard with 2 ml of MIX 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 MIX Diluent to generate 1, 0.5, 0.25, 0.125, 0.0625, 0.03125 ng/ml. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20⁰C.

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

- **Biotinylated rBNP-32 Antibody (100x):** Dilute the antibody 1:100 with MIX Diluent. Spin down the Biotinylated Antibody briefly and only dilute the desired amount of the antibody. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

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°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. Cover wells with a sealing tape and incubate for two 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 completely remove liquid at each step.
- Add 50 µl of Biotinylated rBNP-32 Antibody to each well and incubate for two hours.
- 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.

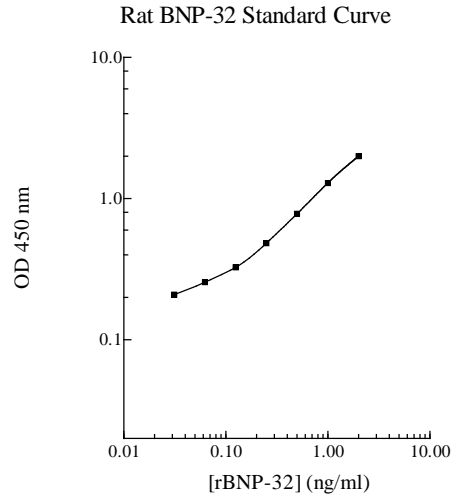
Data Analysis

- 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 4.6 % 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

Version 6.7

Related products

- ERB1202-1 AssayMax Rat BNP-45 ELISA Kit (Plasma, Serum, Tissue and Cell Culture Supernatants samples)