

Datasheet for ABIN612770

BNP-45 ELISA Kit**3** Publications[Go to Product page](#)

Overview

Quantity:	96 tests
Target:	BNP-45
Reactivity:	Rat
Method Type:	Sandwich ELISA
Detection Range:	0.031-1 ng/mL
Minimum Detection Limit:	0.031 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Rat BNP-45 ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of rat BNP-45 in plasma, serum, tissue extract, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures rat BNP45 in less than 5 hours. A polyclonal antibody specific for rat BNP-45 has been pre-coated onto a 96-well microplate with removable strips. The rat BNP-45 in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for rat BNP-45, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.
Brand:	AssayMax™
Sample Type:	Cell Culture Cells, Plasma, Serum, Tissue Lysate
Analytical Method:	Quantitative

Product Details

Detection Method:	Colorimetric
Components:	Rat BNP-45 Microplate: A 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against rat BNP-45. Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay. Rat BNP45 Standard: Rat BNP-45 in a buffered protein base (8 ng, lyophilized). Biotinylated Rat BNP-45 Antibody (50x): A 50-fold biotinylated polyclonal antibody against rat BNP-45 (120 l). 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, 2 bottles). Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (80 l). 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).
Material not included:	Microplate reader capable of measuring absorbance at 405 nm. Pipettes (1-20 µL, 20-200 µL, and multiple channel). Deionized or distilled reagent grade water. Incubator (37 °C)

Target Details

Target:	BNP-45
Abstract:	BNP-45 Products
Background:	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).
Gene ID:	25105
UniProt:	P13205

Application Details

Sample Volume:	50 µL
Assay Time:	5 h
Plate:	Pre-coated
Protocol:	<ul style="list-style-type: none">• Step 1. Add 50 µL of Standard or Sample per well. Incubate 2 hours.• Step 2. Wash, then add 50 µL of Biotinylated Antibody per well. Incubate 2 hours.• Step 3. Wash, then add 50 µL of SP Conjugate per well. Incubate 30 minutes.• Step 4. Wash, then add 50 µL of Chromogen Substrate per well. Incubate 8 minutes.• Step 5. Add 50 µL of Stop Solution per well. Read at 450 nm immediately.
Reagent Preparation:	Freshly dilute all reagents and bring all reagents to room temperature before use. MIX Diluent

Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8 °C. Rat BNP-45 Standard: Reconstitute the 8 ng of Rat BNP-45 Standard with 4 mL of MIX Diluent to generate a 2 ng/mL standard stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard stock solution (2 ng/mL) 1:2 with MIX Diluent to generate 1, 0.5, 0.25, 0.125, 0.0625, and 0.0313 ng/mL solutions. MIX Diluent serves as the zero standard (0 ng/mL). Any remaining solution should be frozen at -20 °C and used within 30 days. 4 Standard Point Dilution [Rat BNP-45] (ng/mL) P1 1 part Standard (2 ng/mL) + 1 part MIX Diluent 1.0000 P2 1 part P1 + 1 part MIX Diluent 0.5000 P3 1 part P2 + 1 part MIX Diluent 0.2500 P4 1 part P3 + 1 part MIX Diluent 0.1250 P5 1 part P4 + 1 part MIX Diluent 0.0625 P6 1 part P5 + 1 part MIX Diluent 0.0313 P7 MIX Diluent 0.0000 Biotinylated Rat BNP-45 Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIX Diluent. Any remaining solution should be frozen at -20 °C. Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. 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.

Sample Collection:	Plasma: Collect plasma using a final concentration of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and assay undiluted plasma for medium and high level of BNP- 45. Samples are recommended for use at 1x or diluted 2x - 10x. Depending on application needs, user should determine proper dilutions. Samples can be stored at -20 °C 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 3000 x g for 10 minutes. Remove serum and perform the assay for medium and high level of BNP-45. Samples are recommended for use at 1x or diluted 2x - 10x. Depending on application needs, user should determine proper dilutions. Samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Cell Culture Supernatants: Centrifuge cell culture media at 3000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20 °C or below. Avoid repeated freeze-thaw cycles. Tissue: Extract tissue samples with 0.1 M phosphate-buffered saline (pH 7.4) containing 1 % Triton X-100 and centrifuge at 14000 x g for 20 minutes. Collect the supernatant, measure the protein concentration, and assay. Freeze the remaining extract at -20 °C or below.
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Assay Procedure:	Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25 °C). Remove
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excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator. Add 50 l of Rat BNP-45 Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition. Wash five times with 200 l of Wash Buffer manually. Invert the plate each time and decant the contents, hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 l of Wash Buffer and then invert the plate, decanting the contents, hit 4-5 times on absorbent material to completely remove the liquid. Add 50 l of Biotinylated Rat BNP-45 Antibody to each well and incubate for 2 hours. Wash the microplate as described above. Add 50 l of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance. Wash the microplate as described above. 5 Add 50 l of Chromogen Substrate per well and incubate for 8 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip. Add 50 l 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. 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.

Calculation of Results:	<ul style="list-style-type: none">• Calculate the mean value of the duplicate or 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 (OD) 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.
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Assay Precision:	Intra-assay and inter-assay coefficients of variation were 4.2 % and 7.8% respectively.
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Restrictions:	For Research Use only
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Handling

Handling Advice:	This product is for Research Use Only and is Not For Use In Diagnostic Procedures. Prepare all reagents (working diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay. Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor. Spin down the SP conjugate vial and the
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Handling

biotinylated antibody vial before opening and using contents. The Stop Solution is an acidic solution. The kit should not be used beyond the expiration date. 2

Storage: 4 °C/-20 °C

Storage Comment: Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date. Store SP Conjugate and Biotinylated Antibody at -20°C. Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C. Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator. Diluent (1x) may be stored for up to 30 days at 2-8°C. Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Publications

Product cited in: Kokk, Kuuslahti, Keisala, Purmonen, Kaipia, Tammela, Orro, Simovart, Pöllänen: "Expression of luteinizing hormone receptors in the mouse penis." in: **Journal of andrology**, Vol. 32, Issue 1, pp. 49-54, (2011) ([PubMed](#)).