MSD® MULTI-ARRAY Assay System

Rat BNP Assay Kit

1-Plate Kit	K153KFD-1
5-Plate Kit	K153KFD-2
25-Plate Kit	K153KFD-4

MSD Toxicology Assays

Rat BNP Assay Kit

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

MESO SCALE DISCOVERY®
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MSD Advantage

MESO SCALE DISCOVERY'S unique spot patterns are a hallmark of our MULTI-ARRAY® technology, which enables the measurement of biomarkers utilizing the next generation of electrochemiluminescent detection. In an MSD assay, specific capture antibodies for the analytes are coated in arrays in each well of a 96-well carbon electrode plate surface. The detection system uses patented SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of the MULTI-ARRAY and MULTI-SPOT® plates. The electrical stimulation is decoupled from the output signal, which is light, to generate assays with minimal background. MSD labels can be conveniently conjugated to biological molecules, are stable, and are non-radioactive. Additionally, only labels near the electrode surface are detected, enabling non-washed assays.

One of the advantages of MSD assays is the minimal sample volume required as compared to a traditional ELISA, which is also limited by its inability to measure more than a single analyte. With an MSD assay, up to ten different biomarkers can be analyzed simultaneously using as little as 10-25 µL of sample. These assays have high sensitivity, up to five logs of linear dynamic range, and excellent performance in complex biological matrices. Combined, these advantages enable the measurement of native levels of biomarkers in normal and diseased samples without multiple dilutions. Further, the simple and rapid protocols of MSD assays provide a powerful tool to generate reproducible and reliable results. The MSD product line offers a diverse menu of assay kits for profiling biomarkers, cell signaling pathways, and other applications, as well as a variety of plates and reagents for assay development.

Introduction

Brain natriuretic peptide (BNP), also known as B-type natriuretic peptide or GC-B, was first isolated from the porcine brain and contains either 26 (BNP-26) or 32 (BNP-32) amino acid residues.[1] In contrast, human and rat BNPs are produced mostly in the heart and the predominant circulating forms consist of 32 and 45 (BNP-45) amino acids respectively. BNP mRNA is not detected in the rat brain, whereas it can be detected in the human brain.[2-4] Thus, there is a substantial difference in the distribution and processing of BNP in different species. The MSD Rat BNP Assay has been optimized for specific and accurate measurement of BNP-45 in serum and plasma samples.

BNP is released into the blood stream when ventricle walls undergo stretch or strain due to increased pressure. Studies have indicated that the levels of BNP are elevated in the plasma of patients with congestive heart failure. Additionally, its levels correlate to the severity of the condition.[5] The measurement of BNP levels can be used in primary care for accurate and rapid diagnosis of heart failure, and for risk stratification of patients suffering from heart attack in emergency care.[6,7] It are also used to assess the effectiveness of treatment for heart failure. Thus, BNP serves as a promising serum and plasma biomarker for cardiac dysfunction and tissue repair.[8,9]

Principle of the Assay

MSD toxicology assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. The assays are available in both singleplex and multiplex formats. In a singleplex assay, an antibody for a specific protein target is coated on one electrode (or "spot") per well. In a multiplex assay, an array of capture antibodies against different targets is patterned on distinct spots in the same well. The Rat BNP Assay is a sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with streptavidin, and the user adds rat BNP capture antibody conjugated to biotin. The user then adds the sample and a solution containing the detection antibody—anti-BNP conjugated with an electrochemiluminescent compound, MSD SULFO-TAG label—over the course of one or more incubation periods. Biotin-conjugated capture antibody binds to the streptavidin that has been immobilized on the working electrode surface, analyte in the sample binds to the capture antibody, and the recruitment of the labeled detection antibody by bound analyte completes the sandwich. The user adds MSD read buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD SECTOR® Imager for analysis. Inside the SECTOR Imager, a voltage applied to the plate electrodes causes the labels bound to the electrode surface to emit light. The instrument measures intensity of emitted light to provide a quantitative measure of BNP present in the sample.

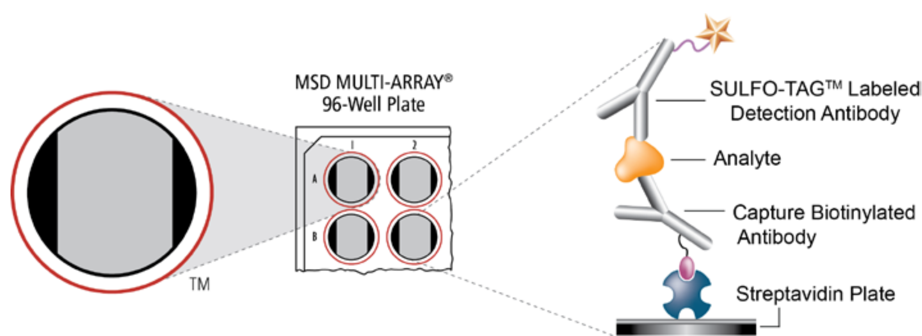


Figure 1. Spot diagram showing placement of analyte capture antibody. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

Reagents Supplied

Product Description	Storage	Quantity per Kit		
		K153KFD-1	K153KFD-2	K153KFD-4
MULTI-ARRAY 96-Well Streptavidin Gold Plate L15SA-1	2–8°C	1 plate	5 plates	25 plates
Anti-rat BNP Biotinylated Capture Antibody (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
SULFO-TAG Anti-rat BNP Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Rat BNP Calibrator (20X)	≤-70°C	1 vial (20 µL)	5 vials (20 µL ea)	25 vials (20 µL ea)
Diluent 8 R54BA-3 (50 mL)	≤-10°C	1 bottle (50 mL)	2 bottles (50 mL ea)	10 bottles (50 mL ea)
Protease Inhibitor Solution (50X)	≤-10°C	1 vial (1 mL)	3 vials (1 mL ea)	15 vials (1 mL ea)
Blocker A Kit R93AA-2 (250 mL)	RT	1 bottle (250 mL)	1 bottle (250 mL)	5 bottles (250 mL ea)
Read Buffer T (4X) R92TC-3 (50 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)

Required Materials and Equipment - not supplied

- Deionized water for diluting concentrated buffers
- 50 mL tubes for reagent preparation
- 15 mL tubes for reagent preparation
- Microcentrifuge tubes for preparing serial dilutions
- Phosphate buffered saline plus 0.05% Tween-20 (PBS-T) for plate washing
- Appropriate liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL into a 96-well microtiter plate
- Plate washing equipment: automated plate washer or multichannel pipette
- Adhesive plate seals
- Microtiter plate shaker

[1] Some SULFO-TAG conjugated detection antibodies may be light-sensitive, so they should be stored in the dark.

Safety

Safe laboratory practices and personal protective equipment such as gloves, safety glasses, and lab coats should be used at all times during the handling of all kit components. All hazardous samples should be handled and disposed of properly, in accordance with local, state, and federal guidelines.

Reagent Preparation

Bring all reagents to room temperature. Thaw the stock calibrator on ice.

> **Important:** Upon first thaw, separate Diluent 8 into aliquots appropriate to the size of your assay needs. This diluent can go through up to three freeze-thaw cycles without significantly affecting the performance of the assay.

Prepare Blocker A Solution

Follow instructions included with the Blocker A Kit.

Prepare Diluent 8 + 2X Protease Inhibitor

For the BNP assay, samples and calibrators are diluted in Diluent 8 that contains Protease Inhibitor. This additive must be added into the diluent by the user before each assay is carried out.

For one plate, combine:

- ❑ 600 µL of Protease Inhibitor Solution (50X)
- ❑ 14400 µL of Diluent 8

Prepare Capture Antibody Solution

The biotin-conjugated capture antibody is provided as a 50X stock solution. The final concentration of the working capture antibody solution should be at 1X. For each plate used, dilute a 60 µL aliquot of the stock capture antibody into 2940 µL of Diluent 8 + 2X Protease Inhibitor.

Prepare Calibrator and Control Solutions

Calibrator for the Rat BNP Assay is supplied at 20-fold higher concentration than the recommended highest calibrator. An 8-point standard curve is recommended with 3-fold serial dilution steps and a zero calibrator. The stock calibrator should be thawed and kept on ice and then should be added into diluent at room temperature to make the standard curve solutions. For the actual concentration of the calibrator, refer to the certificate of analysis (C of A) supplied with the kit. A copy of the kit-specific C of A can also be found at www.mesoscale.com

To prepare an 8-point standard curve in duplicates:

1) Prepare the highest calibrator by adding 15 µL of the calibrator stock vial to 285 µL of Diluent 8 + 2X Protease Inhibitor. Mix well.
2) Prepare the next calibrator by transferring 80 µL of the diluted calibrator to 160 µL of Diluent 8 + 2X Protease Inhibitor. Mix well. Repeat 3-fold serial dilutions 5 additional times to generate 7 calibrators.
3) The recommended 8th standard is Diluent 8 + 2X Protease Inhibitor (i.e. zero calibrator).

Calibrators should be prepared at room temperature no more than 20 minutes before use.

Dilution of Samples

Serum and plasma samples should be diluted 2-fold or depending on the expected level of analytes in the samples. For each replicate, 50 µL of sample is required. If a different sample dilution is required to get the analyte levels into the detection range, Diluent 8 + 2X Protease Inhibitor should be used to dilute the samples.

Prepare Detection Antibody Solution

The detection antibody is provided as a 50X stock solution. The final concentration of the working detection antibody solution should be at 1X. For each plate used, dilute a 60 µL aliquot of the stock detection antibody into 2940 µL of Diluent 8 + 2X Protease Inhibitor.

Prepare Read Buffer

The Read Buffer T (4X) should be diluted 2-fold in deionized water to make a final concentration of 2X Read Buffer T. Add 10 mL of Read Buffer T (4X) to 10 mL of deionized water for each plate.

Prepare MSD Plate

The plate can be used as delivered; no additional preparation (e.g., pre-wetting) is required. The plate has also been exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies.

Assay Protocol

Notes

1. **Addition of Blocker A Solution:** Dispense 150 µL of Blocker A solution into each well of the MSD plate. Seal the plate with an adhesive plate seal, and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.

Shaking a 96-well MSD MULTI-ARRAY plate typically accelerates capture at the working electrode.

2. **Wash and Addition of Capture Antibody Solution:** Wash the plate 3 times with 300 µL/well of PBS-T. Dispense 25 µL of 1X capture antibody solution into each well of the MSD plate. Seal the plate, and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.

3. **Wash and Addition of the Sample or Calibrator:** Wash the plate 3 times with 300 µL/well of PBS-T. Dispense 50 µL of calibrator or diluted sample into separate wells of the MSD plate. Seal the plate with an adhesive plate seal, and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.

4. **Wash and Addition of the Detection Antibody Solution:** Wash the plate 3 times with 300 µL/well of PBS-T. Dispense 25 µL of 1X detection antibody solution into each well of the MSD plate. Seal the plate, and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.

5. **Wash and Read:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 150 µL of 2X Read Buffer T to each well of the MSD plate. Analyze the plate on the SECTOR Imager. No incubation in read buffer is required before reading the plate.

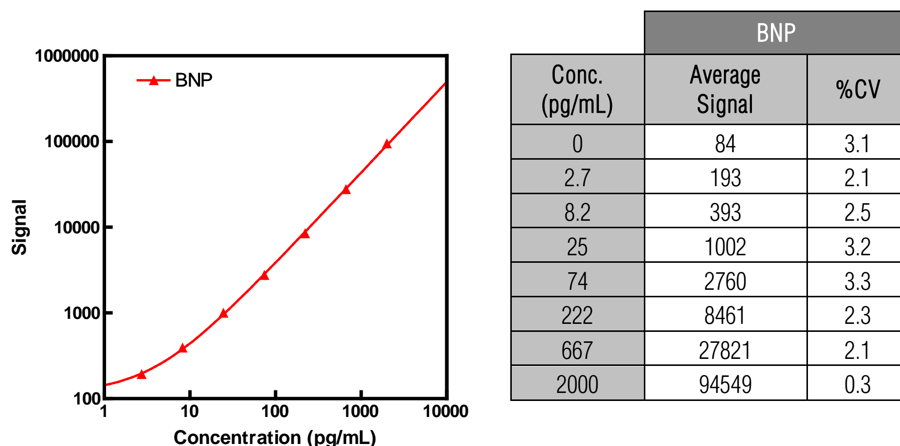
Bubbles in the fluid will interfere with reliable reading of MULTI-ARRAY plate. Use reverse pipetting techniques to ensure bubbles are not created when dispensing the read buffer.

Analysis of Results

The calibrators should be run minimally in duplicate to generate a standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from samples with known levels of the analyte of interest can be used to calculate the concentration of analyte in the sample. The assays have a wide dynamic range (3 - 4 logs) which allows accurate quantification in many samples without the need for dilution. The MSD DISCOVERY WORKBENCH® analysis software utilizes a 4-parameter logistic model (or sigmoidal dose-response) and includes a $1/Y^2$ weighting function. The weighting function is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

Typical Standard Curve

The following standard curve is an example of the dynamic range of the assay. The actual signals may vary, and a standard curve should be run for each set of samples and on each plate for the best quantification of unknown samples.



	BNP	
Conc. (pg/mL)	Average Signal	%CV
0	84	3.1
2.7	193	2.1
8.2	393	2.5
25	1002	3.2
74	2760	3.3
222	8461	2.3
667	27821	2.1
2000	94549	0.3

Sensitivity

The lower limit of detection (LLOD) is the calculated concentration of the signal that is 2.5 standard deviations over the blank (zero calibrator).

	BNP (pg/mL)
LLOD	1.5

Precision

Control samples of high, mid, and low levels were made by spiking calibrator into rat heparin plasma and were measured on each plate.

The controls were run in triplicate on multiple days (n>3).

Average intra-plate %CV is the average %CV of the control replicates within an individual plate.

Inter-plate %CV is the variability of controls across 7 plates over 6 days.

Inter-lot %CV is the variability of controls across 2 kit lots.

	Control	Plates	Average Conc. (pg/mL)	Average Intra-plate %CV	Inter-plate %CV	Inter-lot %CV
BNP	High	14	426	3.6	7.6	7.8
	Mid	14	75	2.7	7.0	7.4
	Low	14	24	4.0	9.0	8.7

Spike Recovery

Normal rat EDTA plasma and heparin plasma were spiked with the calibrator at multiple levels throughout the range of the assay. Spikes were made into neat samples, and then diluted 2-fold.

% Recovery = measured / expected x 100

	BNP			
Sample	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. %CV	% Recovery
EDTA Plasma	0	26	9.0	
	100	131	1.4	104
	200	231	8.0	102
	400	428	11.3	100
	800	881	1.9	107
Heparin Plasma	0	<LLOD	37.4	
	100	105	10.3	104
	200	194	9.1	97
	400	387	3.3	97
	800	846	0.6	106

Linearity

To assess linearity, EDTA plasma and heparin plasma samples were spiked with the calibrator and further diluted 2-fold, 4-fold, 8-fold, 16-fold, 32-fold, and 64-fold prior to testing. The concentrations shown below have been corrected for dilution (concentration = measured x dilution factor). Percent recovery is calculated as the measured concentration divided by the concentration measured from the previous dilution (expected).

% Recovery = (measured x dilution factor) / expected x 100

		BNP		
Sample	Fold Dilution	Conc. (pg/mL)	Conc. %CV	% Recovery
EDTA Plasma	1	701	10.4	
	2	650	9.1	93
	4	589	5.9	91
	8	575	3.1	98
	16	581	1.3	101
	32	589	2.3	101
	64	572	17.2	97
Heparin Plasma	1	561	6.3	
	2	558	10.6	99
	4	553	8.3	99
	8	542	3.0	98
	16	537	1.6	99
	32	569	5.8	106
	64	552	3.6	97

Specificity

Based on the specificity of the capture and detection antibodies, the MSD Rat BNP Assay detects BNP only and does not cross-react with NT-proBNP.

	% Cross-Reactivity	
Assay	BNP Calibrator	NT-proBNP Calibrator
BNP	100	< 0.1
NT-proBNP	< 0.1	100

Samples

Serum, EDTA plasma, and heparin plasma samples collected from normal Sprague-Dawley rats were tested at 2-fold dilution on the Rat BNP Assay. Shown below are the median and range of concentrations for each sample set. Concentrations have been corrected for sample dilution.

Sample	Statistic	BNP
Serum	Median (pg/mL)	2.3
	Range (pg/mL)	2.0-2.5
	N	4
EDTA Plasma	Median (pg/mL)	8.7
	Range (pg/mL)	<LLOD-26
	N	12
Heparin Plasma	Median (pg/mL)	3.7
	Range (pg/mL)	<LLOD-10
	N	3

Assay Components

Calibrator

Synthesized BNP (1-45) peptide is used for this assay.

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
BNP	Mouse Monoclonal	Rabbit Polyclonal

References

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Summary Protocol

MSD 96-well MULTI-ARRAY Rat BNP Assay Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the Rat BNP Assay.

Step 1 : Sample and Reagent Preparation

Bring all reagents to room temperature, and thaw the calibrator on ice.

Prepare capture antibody solution by diluting the 50X capture antibody to 1X in a final volume of 3.0 mL Diluent 8 + 2X Protease Inhibitor per plate.

Prepare an 8-point standard curve using the supplied calibrator:

- The calibrator should be diluted in Diluent 8 + 2X Protease Inhibitor.
- Dilute the stock calibrator 20-fold in Diluent 8 + 2X Protease Inhibitor. Then perform a series of 3-fold dilution steps and prepare a zero calibrator blank.

Prepare detection antibody solution by diluting the 50X detection antibody to 1X in a final volume of 3.0 mL Diluent 8 + 2X Protease Inhibitor per plate.

Prepare 20 mL of 2X Read Buffer T by diluting 4X Read Buffer T with deionized water.

Step 2 : Add Blocker A Solution

Dispense 150 µL/well of Blocker A solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 3 : Wash and Add Capture Antibody Solution

Wash plate 3 times with 300 µL/well of PBS-T.

Dispense 25 µL/well of 1X capture antibody solution into each well of the MSD plate.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 4 : Wash and Add Sample or Calibrator

Wash plate 3 times with 300 µL/well of PBS-T.

Dispense 50 µL/well of calibrator or diluted sample.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 5 : Wash and Add Detection Antibody Solution

Wash plate 3 times with 300 µL/well of PBS-T.

Dispense 25 µL/well of 1X detection antibody solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 6 : Wash and Read Plate

Wash plate 3 times with 300 µL/well of PBS-T.

Dispense 150 µL/well of 2X Read Buffer T.

Analyze plate on SECTOR Imager.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

	1	2	3	4	5	6	7	8	9	10	11	12
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