MSD® MULTI-ARRAY Assay System

Rat NT-proANP Kit

1-Plate Kit	K153MBD-1
5-Plate Kit	K153MBD-2
25-Plate Kit	K153MBD-4

MSD Toxicology Assays

Rat NT-proANP Kit

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Ordering Information

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Introduction

N-terminal proatrial natriuretic peptide (NT-proANP) is the biologically inactive fragment (98 amino acid) of the ANP prohormone. The secreted prohormone is cleaved producing the N-terminus fragment (NT-proANP) and the atrial natruiretic peptide (ANP). The natriuretic peptides have a common characteristic biochemical structure that consists of a ring of 17 amino acids and a disulfide bridge between 2 cysteine molecules.[1]

NT-proANP has been shown to be a useful diagnostic and prognostic tool in heart failure and myocardial infarction.[2] It is more stable and has a longer half-life in circulation than ANP. In patients with mild to moderate cardiac disease, NT-proANP levels were increased in response to atrial wall stress increase. Patients diagnosed with diastolic or systolic dysfunction had 2- to 3-fold higher NT-proANP than the control group.[3] A recent study in rat showed that NT-proANP is an excellent biomarker for cardiac hypertrophy.[4]

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 Rat NT-proANP kit is a sandwich immunoassay (Figure 1). MSD provides a plate pre-coated with capture antibodies. The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) over the course of one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface; recruitment of the detection antibodies by the bound analytes completes the sandwich. The user adds an MSD buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD instrument where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light to provide a quantitative measure of analytes in the sample.

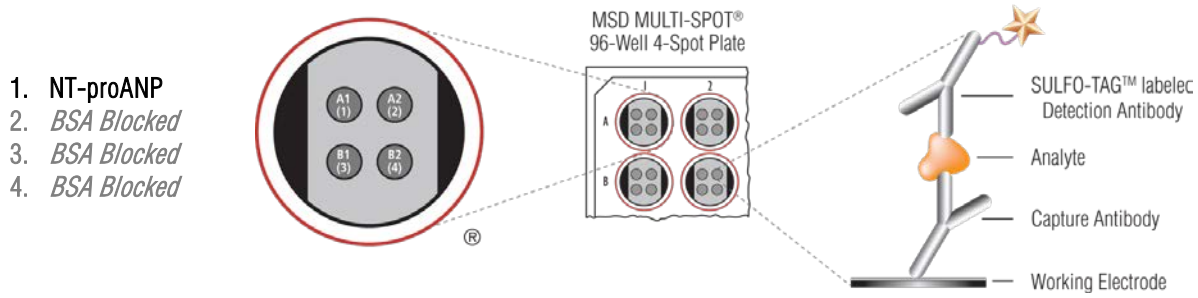


Figure 1. Spot diagram showing placement of analyte capture antibody. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

Reagents Supplied

Product Description	Storage	Quantity per Kit K153MBD-1	K153MBD-2	K153MBD-4
MULTI-SPOT 96-Well 4-Spot Rat NT-proANP Plate N453MBA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-rat NT-proANP Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Rat NT-proANP Calibrator (20X)	≤-70°C	1 vial (20 µL)	5 vials (20 µL ea)	25 vials (20 µL ea)
Diluent 30 R50AB-4 (25 mL)	≤-10°C	1 bottle (25 mL)	5 bottles (25 mL ea)	25 bottles (25 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 Material and Equipment (not supplied)

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

Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines.

[1] SULFO-TAG–conjugated detection antibodies should be stored in the dark.

Reagent Preparation

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

> **Important:** Upon first thaw, separate Diluent 30 into aliquots appropriate for the size of your needs before refreezing.

Prepare Standards

MSD supplies a calibrator for the Rat NT-proANP assay at 20-fold higher concentration than the recommended highest standard. We recommend a 7-point standard curve with 3-fold serial dilution steps and a zero calibrator blank. Signals from the blank should be excluded when generating the curve. Thaw the stock calibrator and keep on ice, then add to diluent at room temperature to make the standard curve solutions. To view the actual concentration of the calibrator, refer to the certificate of analysis (COA) supplied with the kit. You may also find a copy of the lot-specific COA at www.mesoscale.com by entering K153KMD in the search box.

To prepare 7 standard solutions plus a zero calibrator blank for up to 3 replicates:

1) Prepare the highest standard by adding 15 µL of stock calibrator to 285 µL of Diluent 30. Mix well.
2) Prepare the next standard by transferring 100 µL of the highest standard to 200 µL of Diluent 30. Mix well. Repeat 3-fold serial dilutions 5 additional times to generate 7 standards.
3) Use Diluent 30 as the blank.

Dilute Samples

For serum, and plasma samples, MSD recommends a 4-fold dilution in Diluent 30; however, you may adjust dilution factors for the sample set under investigation.

Prepare Detection Antibody Solution

MSD provides the detection antibody in a 50X stock solution. The working detection antibody solution is 1X.

For 1 plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-rat NT-proANP Antibody
- ❑ 2940 µL of Diluent 30

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 2X.

For 1 plate, combine:

- ❑ 10 mL of Read Buffer T (4X)
- ❑ 10 mL of deionized water

You may prepare diluted read buffer in advance and store it at room temperature in a tightly sealed container.

Prepare MSD Plate

MSD plates are pre-coated with capture antibodies (Figure 1) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. Plates can be used as delivered; no additional preparation (e.g., pre-wetting) is required.

Protocol

1. **Add Diluent 30:** Add 25 µL of Diluent 30 to each well. Seal the plate with an adhesive plate seal and incubate for 30 minutes with vigorous shaking (300–1000 rpm) at room temperature.

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

   You may prepare detection antibody solution during incubation.

3. **Wash and Add Detection Antibody Solution:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 25 µL of 1X detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.

   You may prepare diluted read buffer during incubation.

4. **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. Analyze the plate on the MSD instrument. No incubation in read buffer is required before reading the plate.

Notes

Shaking the plate typically accelerates capture at the working electrode.

You may keep excess diluted read buffer in a tightly sealed container at room temperature for later use.

Bubbles introduced when adding read buffer will interfere with imaging of the plate and produce unreliable data. Use reverse pipetting technique to avoid creating bubbles.

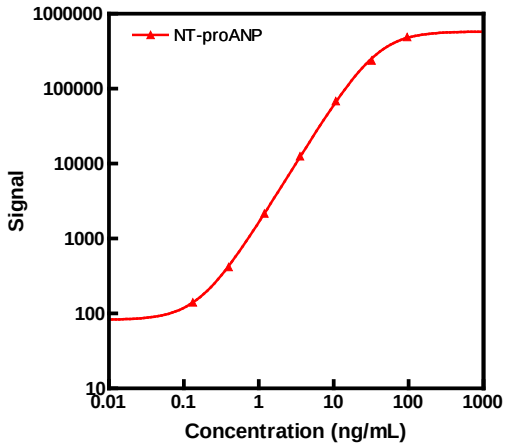
Due to the varying nature of each research application, you should assess assay stability before allowing plates to sit with read buffer for extended periods.

Curve Fitting

MSD DISCOVERY WORKBENCH® software uses least-squares fitting algorithms to generate a standard curve that will be used to calculate the concentration of analyte in the samples. The assays have a wide dynamic range (3–4 logs) that allows accurate quantification without the need for dilution in many cases. The software uses 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 Data

The following standard curve illustrates the dynamic range of the assay. Actual signals will vary. Best quantification of unknown samples will be achieved by generating a standard curve for each plate using a minimum of 2 replicates of standards. For each kit lot, refer to the COA for the actual concentration of the calibrator.



	NT-proANP	
Conc. (ng/mL)	Average Signal	%CV
0	81	4.5
0.13	141	8.3
0.40	417	7.9
1.2	2156	9.7
3.6	12 568	7.2
11	68 143	7.6
32	238 190	5.3
96	491 473	5.6

Sensitivity

The lower limit of detection (LLOD) is a calculated concentration based on a signal 2.5 standard deviations above the background (zero calibrator blank). The LLOD shown below was calculated based on 30 runs.

	NT-proANP
Average LLOD (ng/mL)	0.099

Precision

EDTA plasma-based control samples with high, mid, and low levels of analytes were measured using a minimum of 2 replicates on 9 runs over 3 days.

Average intra-run %CV is the average %CV of the control replicates on an individual run.

Inter-run %CV is the variability of controls across 9 runs.

	Control	Runs	Average Conc. (ng/mL)	Average Intra-run %CV	Inter-run %CV
NT-proANP	High	9	78	15.3	3.4
	Mid	9	9.8	4.7	2.8
	Low	9	0.86	6.2	2.7

Spike Recovery

Normal rat serum and EDTA plasma samples were diluted 4-fold, spiked with calibrators at multiple levels throughout the range of the assay and spiked samples were further diluted 4-fold before testing. The average percent recovery shown below was calculated from samples with values above the LLOD.

% Recovery=measured/expected∗100

	NT-proANP		
Sample Type	Spike Conc. (ng/mL)	Average % Recovery	% Recovery Range
Serum (N=4)	0.247	97	96–99
	0.741	90	78–96
	2.22	94	87–104
	6.67	88	77–99
	20.0	83	78–91
EDTA Plasma (N=6)	0.247	95	91–101
	0.741	94	88–101
	2.22	94	86–102
	6.67	90	83–100
	20.0	82	76–90

Dilution Linearity

To assess linearity, normal rat serum and EDTA plasma samples were diluted 2-fold, 4-fold, 8-fold, 16-fold, and 32-fold before testing. Percent recovery was calculated as the dilution corrected concentration divided by the expected concentration at 4-fold dilution. The average percent recovery shown below was calculated from samples with values above the LLOD.

% Recovery= (measured∗dilution factor)/(measured at 1:4 dilution∗4)∗100

		NT-proANP	
Sample Type	Fold Dilution	Average % Recovery	% Recovery Range
Serum (N=4)	2	101	98–104
	4	100	N/A
	8	92	89–98
	16	83	81–85
	32	83	78–94
EDTA Plasma (N=6)	2	114	112–115
	4	100	N/A
	8	89	87–92
	16	80	74–85
	32	78	71–83

Specificity

MSD Rat NT-proANP assay detects NT-proANP only and does not show any cross-reactivity with NT-proBNP.

Stability

Kit components were tested for freeze-thaw stability. Results (not shown) demonstrated that calibrator can go through 3 freeze-thaw cycles without significantly affecting assay performance. Rat EDTA plasma samples can go through 5 freeze-thaw cycles without any significant changes in their measured concentrations.

Tested Samples

Serum and EDTA plasma samples were collected from normal Sprague-Dawley rats, diluted 4-fold, and tested with the Rat NT-proANP assay. Median and range of concentrations for each sample set are displayed below. Concentrations are corrected for sample dilution.

Sample Type	Statistic	NT-proANP
Serum	Median (ng/mL)	15.1
	Range (ng/mL)	3.89–21.3
	Number of Samples	15
EDTA Plasma	Median (ng/mL)	15.7
	Range (ng/mL)	10.1–39.0
	Number of Samples	27

Assay Components

Calibrator

Full-length recombinant NT-proANP protein is used for this assay.

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
NT-proANP	Mouse Monoclonal	Mouse Monoclonal

References

1. Lijnen P, et al. Natriuretic Peptides in Heart Failure and Post-Myocardial Infarction. Current Hypertension Reviews, 2005,1:7-13
2. Moertl D, et al. Comparison of Midregional Pro-Atrial and B-Type Natriuretic Peptides in Chronic Heart Failure. J Am Coll Cardiol. 2009 May 12; 53(19):1783-90.
3. Hoffmann U, et al. Increased plasma levels of NT-proANP and NT-proBNP as markers of cardiac dysfunction in septic patients. Clin Lab. 2005;51(7-8):373-9.
4. Hall C, et al. N-terminal proatrial natriuretic peptide in primary care: relation to echocardiographic indices of cardiac function in mild to moderate cardiac disease. Int J Cardiol, 2003 Jun;89(2-3):197-205.

Summary Protocol

MSD 96-well MULTI-ARRAY® Rat NT-proANP Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Rat NT-proANP assay.

Sample and Reagent Preparation

- ❑ Bring all reagents to room temperature and thaw the calibrator on ice.
- ❑ Prepare 7 standard solutions using the supplied calibrator:
  - Dilute the stock calibrator 20-fold in Diluent 30.
  - Perform a series of 3-fold dilution steps and prepare a zero calibrator blank.
- ❑ Dilute samples 4-fold in Diluent 30 before adding to the plate.
- ❑ Prepare detection antibody solution by diluting stock detection antibody 50-fold in Diluent 30.
- ❑ Prepare 2X Read Buffer T by diluting stock 4X Read Buffer T 2-fold with deionized water.

Step 1 : Add Diluent 30

- ❑ Add 25 µL/well of Diluent 30.
- ❑ Incubate at room temperature with vigorous shaking (300–1000 rpm) for 30 minutes.

Step 2 : Wash and Add Sample or Calibrator

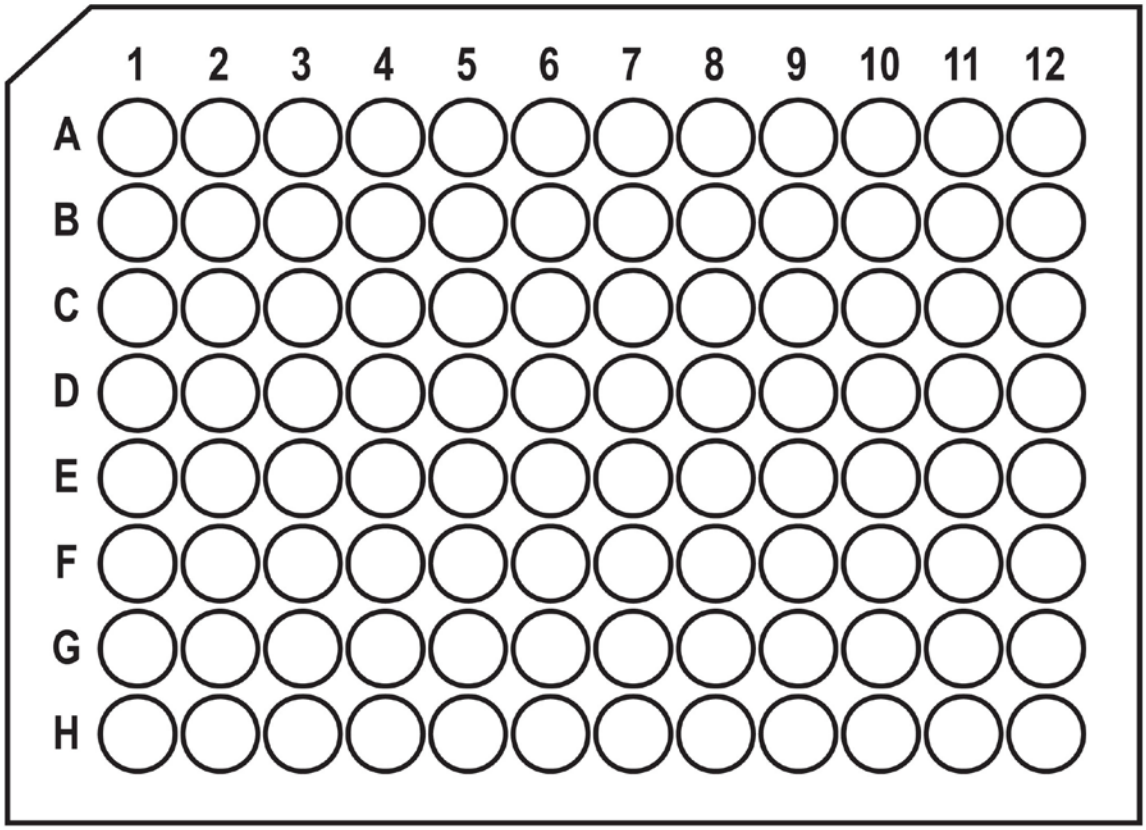
- ❑ Wash plate 3 times with 300 µL/well of PBS-T.
- ❑ Add 50 µL/well of calibrator or diluted sample.
- ❑ Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 3 : Wash and Add Detection Antibody Solution

- ❑ Wash plate 3 times with 300 µL/well of PBS-T.
- ❑ Add 25 µL/well of 1X detection antibody solution.
- ❑ Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 4 : Wash and Read Plate

- ❑ Wash plate 3 times with 300 µL/well of PBS-T.
- ❑ Add 150 µL/well of 2X Read Buffer T.
- ❑ Analyze plate on an MSD instrument.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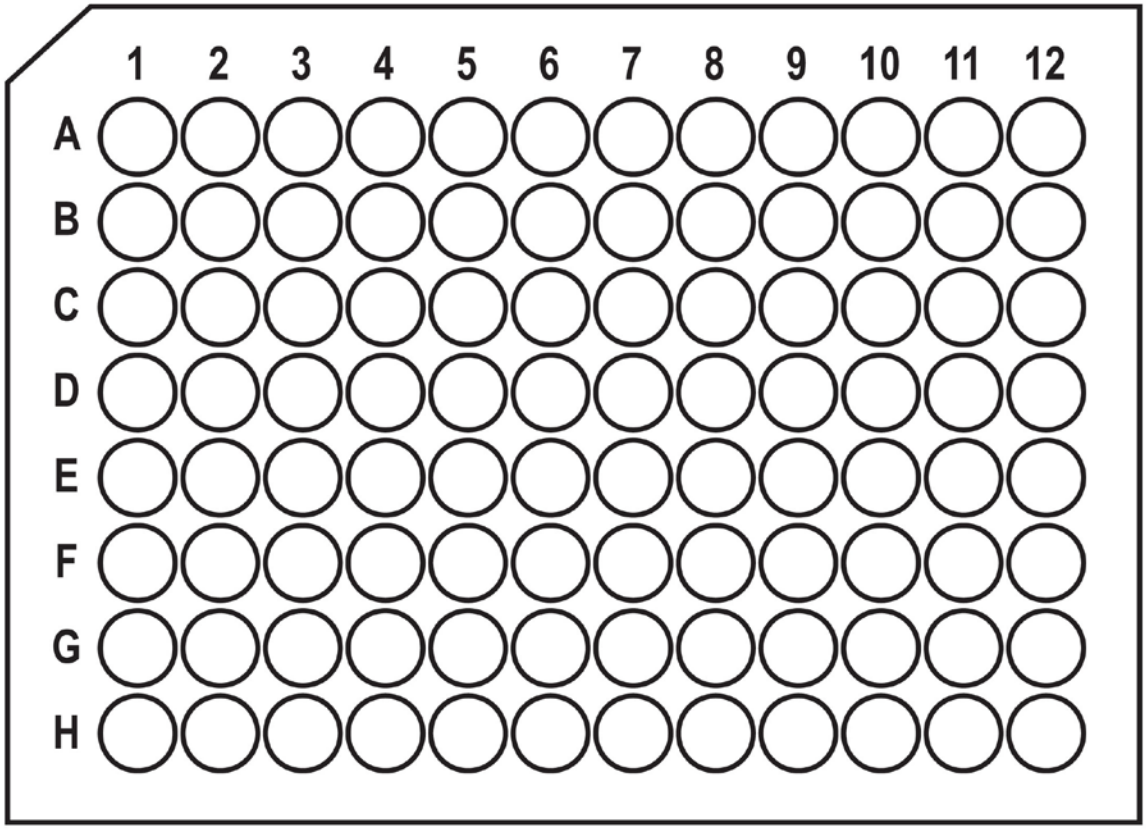
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