

MSD[®] Rat NT-proBNP Assay Kit

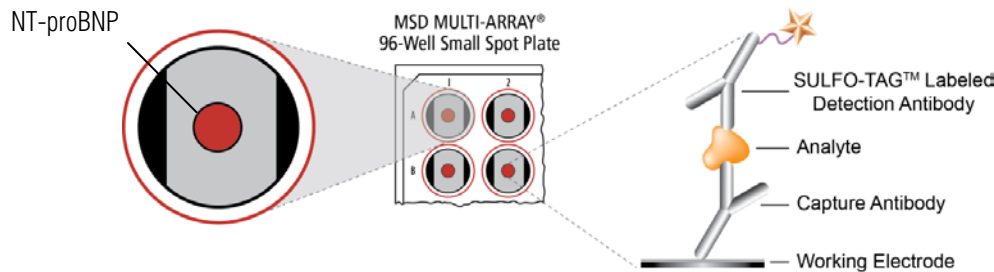
For quantitative determination in rat serum and plasma



Alzheimer's Disease
BioProcess
Cardiac
Cell Signaling
Clinical Immunology
Cytokines
Hypoxia
Immunogenicity
Inflammation
Metabolic
Oncology
Toxicology
Vascular

Catalog Numbers

Rat NT-proBNP Assay Kit	
Kit size	
1 plate	K153JKD-1
5 plates	K153JKD-2
25 plates	K153JKD-4



N-terminal pro-brain (or B-type) natriuretic peptide (NT-proBNP) is produced predominately by the cardiac ventricular myocytes.¹ It is released in response to volume expansion and filling pressure and is involved in maintaining intravascular volume homeostasis.² The generation of NT-proBNP initially starts with the formation of a 134 amino acid (aa) prepro-BNP containing a 26 aa signal sequence. Proteolytic cleavage of the signal peptide releases pro-BNP, which contains 108 aa residues. Further proteolysis of pro-BNP generates a biologically inactive 76 aa NT-proBNP and an active 32 aa BNP molecule.

Elevated plasma levels of BNP and NT-proBNP have been observed at times of cardiac stress and damage. Hence, they are widely used as a diagnostic tool for the occurrence and severity of heart failure and coronary syndrome.³⁻⁵ Measurement of NP levels may help in risk stratification of patients suffering from heart attack in emergency care and in accurate and rapid diagnosis of heart failure in primary care.

The MSD Rat NT-proBNP Assay is available on 96-well plates. This datasheet outlines the performance of the assay.

Ordering information

MSD Customer Service
Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

Assay Sensitivity

	NT-proBNP (pg/mL)
LLOD	0.74

The lower limit of detection (LLOD) is measured as the concentration at 2.5 standard deviations over the background.

Company Address

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MSD Advantage

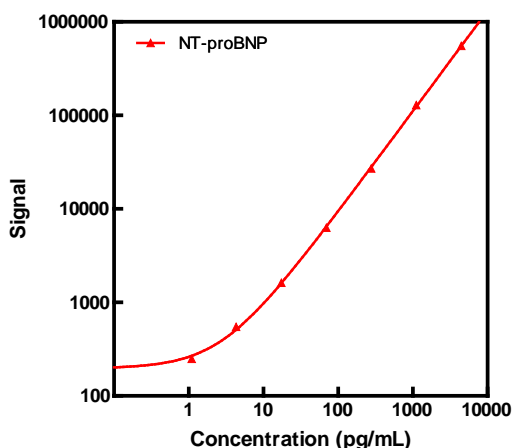
- **Multiplexing:** Multiple analytes can be measured in one well using typical sample volumes of 25 μ L or less without compromising speed or performance
- **Large dynamic range:** Linear range of up to five logs enables the measurement of native levels of biomarkers in normal and diseased samples without multiple dilutions
- **Minimal background:** The stimulation mechanism (electricity) is decoupled from the signal (light)
- **Simple protocols:** Only labels near the electrode surface are detected, enabling assays with fewer washes
- **Flexibility:** Labels are stable, non-radioactive, and conveniently conjugated to biological molecules
- **High sensitivity and precision:** Multiple excitation cycles of each label enhance light levels and improve sensitivity

For a complete list of products, please visit our website at www.mesoscale.com.

MSD Toxicology Assays

Typical Standard Curve:

The following standard curve is an example of the dynamic range of the Rat NT-proBNP Assay.



Conc. (pg/mL)	NT-proBNP	
	Average Signal	%CV
0	170	11.9
1.1	249	6.8
4.3	547	5.6
17	1622	3.6
69	6292	7.8
278	27010	6.3
1111	128604	2.5
4445	554796	4.5

Spike Recovery:

Normal rat EDTA plasma and heparin plasma were spiked with the calibrator at multiple levels throughout the range of the assay. The samples were diluted 4-fold and then spiked with calibrator at the levels indicated in the table below.

% Recovery = measured / expected x 100

Sample	NT-proBNP			% Recovery
	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. %CV	
EDTA Plasma	0	34	1.9	
	8.2	45	2.1	107
	25	65	1.1	111
	74	124	2.3	115
	222	313	2.9	122
	667	855	3.2	122
	2000	2457	2.1	121
Heparin Plasma	0	9.0	4.7	
	1.0	9.3	6.6	94
	3.9	13	2.2	101
	16	25	1.5	101
	63	72	7.9	101
	250	243	1.3	94
	1000	849	1.0	84
	4000	3407	0.7	85

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MSD Toxicology Assays

Linearity:

To assess linearity, EDTA plasma and heparin plasma samples were diluted 2-fold, 5-fold, 10-fold, 20-fold and 40-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).

$$\% \text{ Recovery} = (\text{measured} \times \text{dilution factor}) / \text{expected} \times 100$$

Sample	Fold Dilution	NT-proBNP		
		Conc. (pg/mL)	Conc. %CV	% Recovery
EDTA Plasma	2	97	5.6	
	5	78	3.6	81
	10	74	6.7	95
Heparin Plasma	2	138	5.7	
	5	109	0.9	79
	10	127	4.2	117
	20	148	7.7	116

Precision:

Control samples of high, mid, and low levels were made by spiking calibrator into rat EDTA 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 9 plates over 8 days.

	Control	Plates	Average Conc. (pg/mL)	Average Intra-plate %CV	Inter-plate %CV
NT-proBNP	High	9	2268	4.6	7.4
	Mid	9	243	3.7	6.6
	Low	9	18	4.2	14.8

Samples:

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

Sample	Statistic	NT-proBNP
Serum	Median (pg/mL)	4.5
	Range (pg/mL)	<LLOD-9.8
	N	8
EDTA Plasma	Median (pg/mL)	52
	Range (pg/mL)	31-130
	N	8
Heparin Plasma	Median (pg/mL)	45
	Range (pg/mL)	19-132
	N	8

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Cited References:

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4. Cowie MR, Struthers AD, Wood DA, Coats AS, Thompson SG, PooleWilson PA, Sutton GC. Value of natriuretic peptides in assessment of patients with possible new heart failure in primary care. *Lancet.* 1997 Nov 8;350(9088):1349-53.
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References using MSD's platform for the measurement of toxicology biomarkers:

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8. Tonomura Y, Mori Y, Torii M, Uehara T. Evaluation of the usefulness of biomarkers for cardiac and skeletal myotoxicity in rats. *Toxicology.* 2009 Dec 21;266(1-3):48-54. Epub 2009 Oct 23.

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