Rat NT-proBNP ELISA

for the quantitative determination of Rat N-terminal pro-brain natriuretic peptide (NT-proBNP) in serum or plasma

Cat. No. BI-1204R, 12 x 8 tests

FOR RESEARCH USE ONLY

CONTENTS

ASSAY CHARACTERISTICS Summary

Method	Sandwich ELISA, HRP/TMB, 12x8-well detachable strips			
Sample type	Serum and plasma			
Sample volume	10 µl / well			
Assay time	2 hours / 1 hour / 30 min			
Sensitivity	LOD: 21pg/ml, LLOQ: 50pg/ml			
Standard range	0 – 3200 pg/ml (0 / 100 / 200 / 400 / 800 / 1600 / 3200)			
Precision		**n**	**Average %CV**	
	Within-run	3	≤6	
	In-between-run	9	≤4	
Accuracy		**n**	**Average %recovery**	
			+1600 pg/ml	**+400 pg/ml**
	Rat serum	6	90	86
	Rat plasma	3	88	81
Dilution linearity of endogenous rat NT-proBNP		**n**	**Average % of expected dilution**	
			1+1	**1+3**	**1+7**
	Rat serum	5	106	116	113
Specificity	Endogenous and recombinant rat NT-proBNP			
Use	Research use only			
Values of healthy animals*		**n**	**Median NT-proBNP (pg/ml)**	
	Rat serum	6	74	
	Rat plasma	14	22	
Values in CTRL group - disease model AN	**Rat serum**	5	379	
Values in disease model AN	**Rat serum**	8	706	
Values in CTRL group - disease model RMR	**Rat serum**	4	110	
Values in disease model RMR	**Rat serum**	7	708	

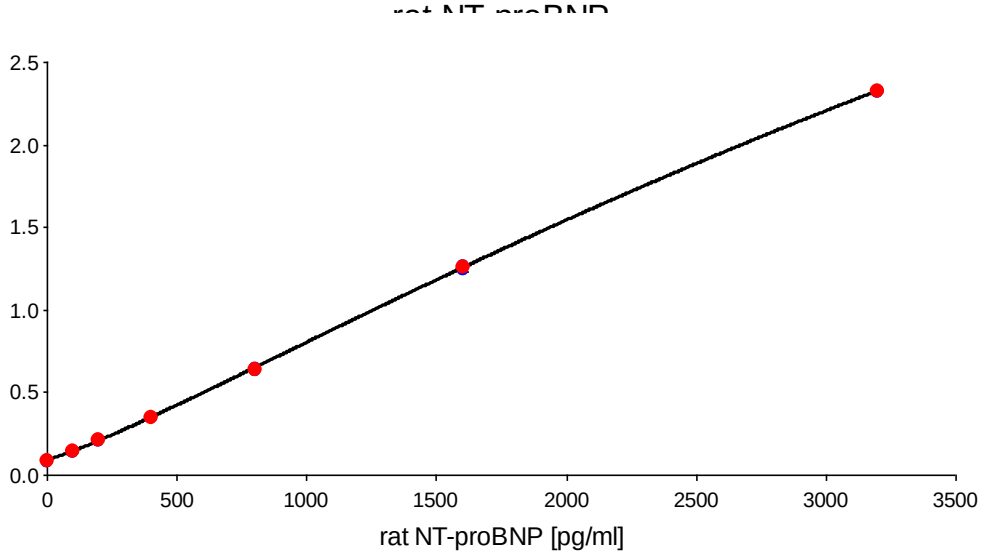
** values derive from from rat strains with different genetic backgrounds*
Abbreviations: CTRL: control; AD: adriamycin nephropathy; RMR: renal mass reduction

PRODUCT OVERVIEW

The Rat NT-proBNP immunoassay is a 3.5 hour, 96-well sandwich ELISA for the quantitative determination of rat NT-proBNP in serum or plasma.

TYPICAL STANDARD CURVE

The figure below shows a typical standard curve for the Rat NT-proBNP ELISA. The immunoassay is calibrated against recombinant rat NT-proBNP peptide.



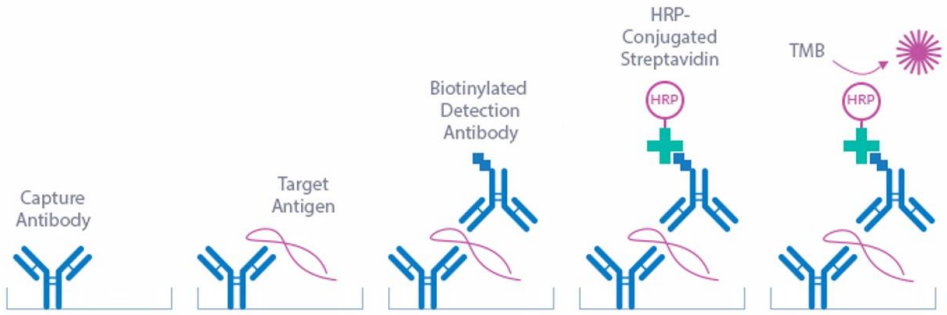
CALIBRATION

The Rat NT-proBNP immunoassay is calibrated against recombinant rat NT-proBNP peptide.

PRINCIPLE OF THE ASSAY

The Rat NT-proBNP ELISA kit is a sandwich enzyme immunoassay for the quantitative determination of NT-proBNP in rat samples.

Figure explaining the principle of a sandwich ELISA:



Target antigen: rat NT-proBNP
Calibrator: recombinant rat NT-proBNP

This kit is a sandwich enzyme immunoassay for the quantitative determination of rat NT-proBNP in serum and plasma samples. In a first step, STD/CTRL/Sample (Standard, Control, Sample) and detection antibody (biotinylated polyclonal anti-rat NT-proBNP antibody, AB) are pipetted into the respective wells of the microtiter strips, which are pre-coated with a polyclonal anti-rat NT-proBNP antibody. Rat NT-proBNP present in the sample binds to the pre-coated antibody in the well and forms a sandwich with the detection antibody.

After a first wash step, which removes non-specifically unbound material, the conjugate (Streptavidin-HRPO) is added and reacts with the detection antibody. After another washing step, the substrate (TMB, tetramethylbenzidine) is pipetted into the wells.

The enzyme catalysed color change of the substrate is directly proportional to the amount of rat NT-proBNP present in the sample. This color change is detectable with a standard microtiter plate ELISA reader. The concentration of rat NT-proBNP in the sample is determined directly from the dose response curve. The kit utilizes recombinant rat NT-proBNP as a calibrator.

SAMPLE VALUES

Rat NT-proBNP Values in Apparently Healthy Animals

To provide an estimation of rat NT-proBNP values from apparently healthy rats we measured a small sample number of rat serum/plasma samples deriving from rat strains with different genetic backgrounds. Rat NT-proBNP reference values may differ from other rat strains. A summary of the results is shown below:

		Rat NT-proBNP (pg/ml)				Detectable (%)
Sample matrix	**n***	**Mean**	**Median**	**Minimum**	**Maximum**	
Serum	6	82	74	0	169	83
Plasma **	14	47	22	0	185	86

** samples derived from rat strains with different genetic backgrounds.*
Rat NT-proBNP reference values may differ from other rat strains.
***EDTA and Citrate plasma*

It is recommended to establish the normal range for each laboratory.

Rat NT-proBNP Values in a Clinical Cohort (rat models)

Rat NT-proBNP levels were measured in rats from the respective control groups and in rats from kidney disease models adriamycin nephropathy (AN) and renal mass reduction (RMR).

Summary of rat NT-proBNP values in rat kidney disease models (control group /AN)

		Rat NT-proBNP (pg/ml)			
Cohort	**n**	**Mean**	**Median**	**Minimum**	**Maximum**
Control group	5	335	379	270	405
AN	8	712	706	490	922

Detailed data:

ID	Rat NT-proBNP (pg/ml)	
	Control group	AN
#1	384	788
#2	379	623
#3	270	490
#4	405	688
#5	237	840
#6	n.a.	922
#7	n.a.	723
#8	n.a.	624

n.a.=not available

Summary of rat NT-proBNP values in rat kidney disease models (control group/RMR):

		Rat NT-proBNP (pg/ml)			
Cohort	**n**	**Mean**	**Median**	**Minimum**	**Maximum**
Control group	4	108	110	45	169
RMR	7	767	708	275	1724

Detailed data:

ID	Rat NT-proBNP (pg/ml)	
	Control group	RMR
#1	90	275
#2	129	587
#3	45	797
#4	169	814
#5	n.a.	708
#6	n.a.	463
#7	n.a.	1724

n.a.=not available

ASSAY PERFORMANCE CHARACTERISTICS

ACCURACY

The accuracy of an ELISA is defined as the precision with which it can recover samples of known concentrations.

The recovery of the rat NT-proBNP ELISA was measured by adding recombinant rat NT-proBNP into samples with known concentration of endogenous rat NT-proBNP. The %recovery of the spiked concentration was calculated as the percentage of measured compared over the expected value. All our ELISAs are expected to have %recovery rates within 20% of the nominal value of the sample.

Sample matrix	n	Mean %Recovery	
		+1600 pg/ml	+400 pg/ml
Serum	6	90	86
Plasma	3	88	81

Detailed data showing recovery of recombinant rat NT-proBNP in serum samples:

	Rat NT-proBNP			% Recovery	
Sample ID	**Reference**	**+1600 pg/ml**	**+400 pg/ml**	**+1600 pg/ml**	**+400 pg/ml**
Serum 1	161	1631	521	97	95
Serum 2	800	1724	1017	83	79
Serum 3	388	1707	704	95	91
Serum 4	289	1533	572	87	80
Serum 5	198	1533	496	90	81
Serum 6	479	1663	779	89	90
			Mean	**90**	**86**
			Min	83	79
			Max	97	95

Detailed data showing recovery of recombinant rat NT-proBNP in plasma samples:

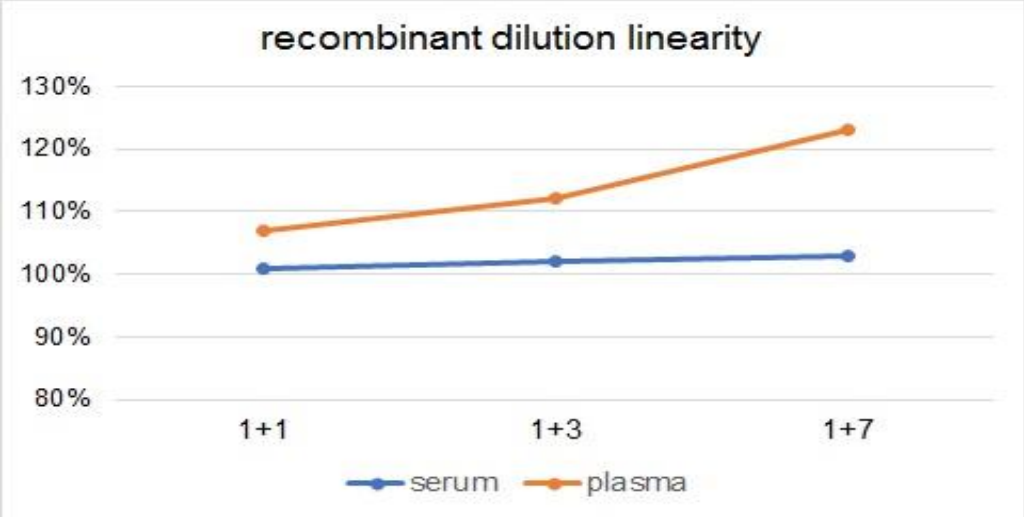
	Rat NT-proBNP			% Recovery	
Sample ID	**Reference**	**+1600 pg/ml**	**+400 pg/ml**	**+1600 pg/ml**	**+400 pg/ml**
Plasma 1	185	1441	485	84	81
Plasma 2	118	1562	417	94	78
Plasma 3	78	1423	400	87	83
			Mean	**88**	**81**
			Min	84	78
			Max	94	83

DILUTION LINEARITY & PARALLELISM

Tests of dilution linearity and parallelism ensure that both endogenous and recombinant samples containing rat NT-proBNP behave in a dose dependent manner and are not affected by matrix effects. Dilution linearity assesses the accuracy of measurements in diluted samples spiked with known concentrations of recombinant analyte. By contrast, parallelism refers to dilution linearity in samples and provides evidence that the endogenous analyte behaves in same way as the recombinant one.

Dilution linearity was assessed by serially diluting samples spiked with 1600 pg/ml *recombinant rat NT-proBNP* with assay buffer.

The figure and table below show the mean recovery and range of serially diluted recombinant rat NT-proBNP in serum and plasma samples:



		% Recovery of recombinant rat NT-proBNP in diluted samples					
Sample matrix	**n**	**1+1**		**1+3**		**1+7**	
		Mean	**Range**	**Mean**	**Range**	**Mean**	**Range**
Serum	3	101	99-103	102	99-103	103	93-108
Plasma	2	107	98-116	112	103-121	123	112-135

Experiments:
Data showing dilution linearity of 1600 pg/ml recombinant rat NT-proBNP spiked into *serum* samples (ref) containing endogenous rat NT-proBNP:

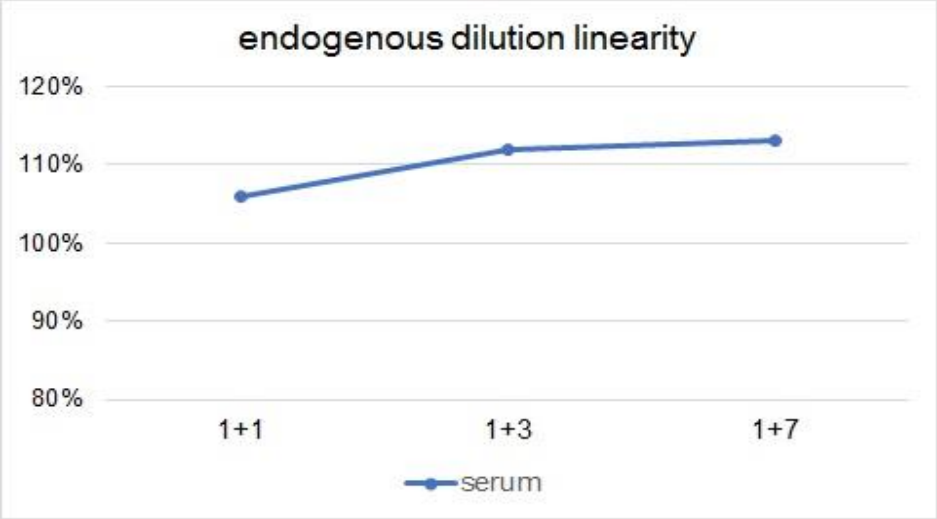
	Rat NT-proBNP (pg/ml)				% Recovery		
Sample ID	**Ref +1600 pg/ml**	**1+1**	**1+3**	**1+7**	**1+1**	**1+3**	**1+7**
Serum 1	1912	973	494	254	102	103	106
Serum 2	1671	825	415	195	99	99	93
Serum 3	1739	899	449	235	103	103	108
				Mean	**101**	**102**	**103**
				Min	99	99	93
				Max	103	103	108

Data showing dilution linearity of 1600 pg/ml recombinant rat NT-proBNP spiked into *plasma* samples (ref) containing endogenous rat NT-proBNP:

	Rat NT-proBNP (pg/ml)				% Recovery		
Sample ID	**Ref +1600 pg/ml**	**1+1**	**1+3**	**1+7**	**1+1**	**1+3**	**1+7**
Plasma 1	1441	835	435	243	116	121	135
Plasma 2	1562	768	404	218	98	103	112
					107	**112**	**123**

Parallelism was assessed by serially diluting samples containing *endogenous rat NT-proBNP* with assay buffer.

The figure and table below show the mean recovery and range of serially diluted endogenous rat NT-proBNP in serum samples:



		% Recovery of endogenous rat NT-proBNP in diluted samples					
Sample matrix	**n**	**1+1**		**1+3**		**1+7**	
		Mean	**Range**	**Mean**	**Range**	**Mean**	**Range**
Serum	5	106	100-112	116	108-125	113	97-119

Experiments:
Data showing dilution linearity of *endogeneous rat NT-proBNP* in *serum* samples:

Sample ID	**Rat NT-proBNP (pg/ml)**				**% Recovery**		
	Reference	**1+1**	**1+3**	**1+7**	**1+1**	**1+3**	**1+7**
Serum 1	1240	665	386	185	107	125	119
Serum 2	838	417	236	120	100	113	115
Serum 3	902	503	278	130	112	123	115
Serum 4	1119	579	303	136	103	108	97
Serum 5	672	348	186	100	104	111	119
				Mean	**105**	**116**	**113**
				Min	100	108	97
				Max	112	125	119

PRECISION

The precision of an ELISA is defined as its ability to measure the same concentration consistently within the same experiments carried out by one operator (within-run precision or repeatability) and across several experiments using the same samples but conducted by several operators using different ELISA lots (in-between-run precision or reproducibility).

Within-Run Precision (repeatability or intra-assay precision)

Within-run / intra-assay precision was assessed by measuring 6 samples of known concentrations 3 times within 1 Rat NT-proBNP ELISA kit lot by 1 operator.

Intra-assay (n=3)	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Mean (pg/ml)	195	1600	350	153	532	1686
SD (pg/ml)	4.7	27.0	14.8	9.8	15.9	87.9
CV (%)	2	2	4	6	3	5

In-Between-Run Precision (reproducibility or inter-assay precision)

In-between-run / inter-assay precision was assessed by measuring 2 samples 9 times within different Rat NT-proBNP ELISA kit lots by 2 different operators.

Inter-assay (n=9)	Sample 1	Sample 2
Mean (pg/ml)	199	1603
SD (pg/ml)	8.8	34.4
CV (%)	4	2

DETECTION LIMIT & SENSITIVITY

To determine the sensitivity of the Rat NT-proBNP ELISA, experiments measuring the Lower Limit of Detection (LOD) and the Lower Limit of Quantification (LLOQ) were conducted.

The LOD, also called the detection limit, is the lowest point at which a signal can be distinguished from the background signal. It is defined as the mean back calculated concentration of standard 1 (0 pg/ml of rat NT-proBNP, five independent measurements) plus three times the standard deviation of the measurements.

The LLOQ, or sensitivity of an assay, is the lowest concentration at which an analyte can be accurately quantified. The criteria for accurate quantification at the LLOQ are an analyte recovery between 75% and 125% and a coefficient of variation (CV) of less than 25%. To determine the LLOQ, standard 2, i.e. the lowest standard containing the rat NT-proBNP, is diluted, measured five times and its concentration is back calculated. The lowest dilution, which meets both criteria, is reported as the LLOQ.

The following values were determined for the Rat NT-proBNP ELISA:

LOD	**21.4 pg/ml**
LLOQ	**50 pg/ml**

SAMPLE STABILITY

Freeze-thaw experiments:

The stability of endogenous rat NT-proBNP was tested by comparing rat NT-proBNP concentrations that were measured in samples that had undergone 5 freeze-thaw cycles.

	Rat NT-proBNP [OD]				% Recovery after 5 freeze/thaw cycles
Sample ID	**Reference**	**1x**	**3x**	**5x**	
Serum 1	0.377	0.411	0.406	0.395	105
Serum 2	0.277	0.282	0.288	0.298	108
Serum 3	0.332	0.358	0.352	0.358	108
Serum 4	0.703	0.730	0.731	0.759	108
				Mean	**107**

Rat serum samples can undergo up to 5 freeze-thaw cycles.

Benchtop stability experiments:

The stability of endogenous rat NT-proBNP was determined by comparing rat NT-proBNP concentrations of samples tested for bench top stability (1h and 3h at room temperature).

	Rat NT-proBNP [OD]			% Recovery	
Sample ID	**Reference**	**1h/RT**	**3h/RT**	**1h/RT**	**3h/RT**
Serum 1	0.377	0.392	0.395	104	105
Serum 2	0.277	0.277	0.285	100	103
Serum 3	0.332	0.380	0.352	114	106
Serum 4	0.703	0.743	0.757	106	108
			Mean	106	105

Samples are stable up to 3h at room temperature.

STANDARD STABILITY

Freeze-thaw experiments:

The stability of recombinant rat NT-proBNP was tested by comparing 3 measurements of standards (employed in the kit) spiked to different rat NT-proBNP values that had undergone 5 freeze-thaw cycles. The mean recovery of the STD4 and STD7 after 5 freeze-thaw cycles is 101%.

	Rat NT-proBNP [OD]				% Recovery after 5 freeze/thaw cycles
Sample ID	**Reference**	**1x**	**3x**	**5x**	
STD4	0,454	0,461	0,455	0,473	104
STD7	3,348	3,416	2,978	3,257	97
				Mean	**101**

Standards can undergo up to 5 freeze-thaw cycles.

Benchtop stability experiments

The stability of recombinant rat NT-proBNP was determined by comparing rat NT-proBNP concentrations of standards (employed in the kit) tested for bench top stability for 1h and 3h at room temperature and 20h at +4°C. The mean recovery of standard concentrations after 3h at room temperature and 20h at 4°C is 97% and 95%, respectively.

	Rat NT-proBNP [OD]				% Recovery		
Sample ID	**Reference**	**1h/RT**	**3h/RT**	**20h/+4°C**	**1h/RT**	**3h/RT**	**20h/+4°C**
STD4	0,454	0,433	0,464	0,454	95	102	100
STD7	3,348	3,038	3,106	3,043	91	93	91
				Mean	**93**	**97**	**95**

SPECIFICITY

The specificity of an ELISA is defined as its ability to exclusively recognize the analyte of interest.

COMPETITION OF SIGNAL

Competition experiments were carried by adding 25-fold surplus of unlabelled capture antibody during the sample incubation step. The concentration measured in this mixture was then compared to a reference value, which was obtained from the same sample but without the incubation with un-labelled capture antibody.

	Rat NT-proBNP c[pg/ml]		% Competition*
Sample ID	**Reference**	**Reference + rat NT-proBNP coating antibody**	
recombinant STD7	3200	3	100
Serum 1	515	0	100
Serum 2	542	4	99
Serum 3	340	0	100
Serum 4	1120	0	100
Serum 5	142	0	100
Serum 6	519	0	100
Serum 7	1100	0	100
		Mean	**100**

**calculation competition = [1 - (competed calculated c[pg/ml] of sample / non-competed calculated c[pg/ml] of sample)] x 100*

Additional Documents Available Online (www.bmgrp.com)

Instruction for Use (IFU, protocol booklet)
Material Safety Data Sheet (MSDS)