

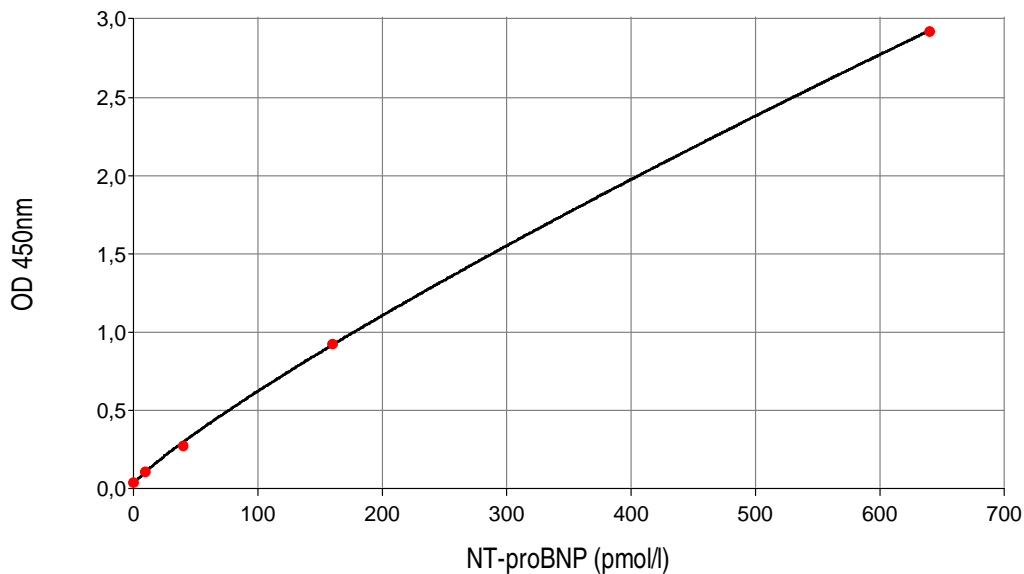
NT-proBNP ELISA (Cat.No. SK-1204)

For the Determination of NT-proBNP (1-76) in Human Samples

ASSAY CHARACTERISTICS

Method	Sandwich ELISA, HRP/TMB, 12x8-well strips
Antibodies/Standard	Capture antibody: polyclonal anti human NT-proBNP antibody, specifically binding to amino acids 31-57 of proBNP. Detection antibody: polyclonal anti human NT-proBNP antibody, conjugated to peroxidase, specifically binding to amino acids 8-29 of proBNP. Standard material: synthetic human NT-proBNP (1-76).
Sample type	Human serum
Standard range	0-640 pmol/l (0/10/40/160/640 pmol/l)
Conversion factor	1 pmol/l = 8.475 pg/ml – refers to NT-proBNP (1-76) that is detected by the ELISA
Sample volume	50 µl / test – direct measurement
Detection limit LOQ	3.0 pmol/l
The lower limit of quantification LLOQ	3.3 pmol/l
Incubation	3 h / 30 min – room temperature

Typical standard curve of Biomedica NT-proBNP ELISA:



Serum values from apparently healthy donors:

Serum (n=70)	NT-proBNP (pmol/l)
Median	5.8
Mean	9.8
Maximum	63.9
Minimum	1.1
Percentil 95%	32.5
Percentil 5%	2.0

Serum values from unselected hospital panel:

Serum (n=117)	NT-proBNP (pmol/l)
Median	46.5
Mean	84.5
Maximum	621.1
Minimum	0.0
Percentil 95%	22.1
Percentil 5%	0.8

Serum values of a heart failure panel NYHA classification II-IV measured with the Biomedica NT-proBNP ELISA (#SK-1204)

n	62
mean	54.0 pmol/l
max	399.3 pmol/l
min	1.1 pmol/l

NYHA classification subgroups:

measured with the Biomedica NT-proBNP ELISA (#SK-1204)

Data on serum values of a heart failure panel **NYHA classification II**

n	41
mean	25.5 pmol/l
max	75.6 pmol/l
min	1.1 pmol/l

Data on serum values of a heart failure panel **NYHA classification III**

n	14
mean	63.2 pmol/l
max	264.3 pmol/l
min	9.2 pmol/l

Data on serum values of a heart failure panel **NYHA classification IV**

n	7
mean	166.8 pmol/l
max	399.3 pmol/l
min	84.1 pmol/l

PERFORMANCE CHARACTERISTICS

Spike Recovery:

The mean recovery of rec. NT-proBNP in serum samples (n=4) is 108%.

Experiment:

Recovery of spiked samples was tested by adding 2 concentrations of human recombinant NT-proBNP (1-76) to 4 different human serum samples.

Data showing spike/recovery of human serum samples:

Matrix	Spike (pmol/l)	Serum			S/R (%)	
		0	80	320	80	320
Sample ID	#1	1.6	88.5	347.2	109	108
	#2	0.1	74.2	360.2	93	113
	#3	1.7	80.9	338.7	99	105
	#4	4.4	81.6	346.5	96	107
Mean (%)					99	108

Dilution Linearity:

Serum (n=3): 112-119% (spiked with recombinant NT-proBNP)

79-86% (endogenous NT-proBNP)

Experiment 1:

Dilution linearity was assessed by spiking serum samples with recombinant NT-proBNP (1-76) (320 pmol/l) and by diluting the samples with STD1 (serum matrix containing 0 pmol/l NT-proBNP, provided in the kit):

Data showing the dilution of recombinant NT-proBNP (1-76) in serum samples:

Serum Sample ID	Reference NT-proBNP (pmol/l)	Spiked NT-proBNP (pmol/l)	Dilution 1+1	
			NT-proBNP (pmol/l)	R (%)
#1	1.7	338.7	202.2	119
#2	0.1	360.2	202.4	112
#3	8.0	278.5	165.7	119
Mean (%)			117	

Experiment 2:

Dilution linearity was assessed by diluting serum samples containing endogenous NT-proBNP with STD1 (serum matrix containing 0 pmol/l NT-proBNP, provided in the kit).

Data showing the dilution of endogenous NT-proBNP (1-76) in serum samples:

Serum Sample ID	Reference NT-proBNP (pmol/l)	Dilution 1+1	
		NT-proBNP (pmol/l)	R (%)
#1	44.2	17.4	79
#2	51.3	20.4	80
#3	61.7	26.5	86
Mean (%)		82	

Intra-assay precision & Inter-assay precision:**Intra-assay (n=6) ≤ 4%, Inter-assay (n=8) ≤ 7%**Experiment:

Intra-assay: 2 samples of known concentrations were tested 3 times in 1 assay by 1 operator.

Inter-assay: 2 samples of known concentrations were tested 8 times in 2 assays by different operators.

Data showing intra-assay and inter-assay precision:

Intra-assay (n=3)	Sample 1	Sample 2	Inter-assay (n=8)	Sample 1	Sample 2
Mean (pmol/l)	60.2	35.2	Mean (pmol/l)	52.1	108.1
SD (pmol/l)	2.0	0.9	SD (pmol/l)	1.7	7.9
CV (%)	4	3	CV (%)	3	7

The limit of quantification (LOQ):

The LOQ is defined as the mean value of the back calculated concentration plus 3 times the standard deviation. The LOQ of the NT-proBNP ELISA is 3 pmol/l.

The lower limit of quantification (LLOQ):

The lower limit of quantification is defined as the accuracy of the back calculated concentrations and shall not exceed ±25% (acc. to ICH [Ref. 1]).

For the NT-proBNP ELISA the LLOQ was determined as 3.3 pmol/l.

Freeze/thaw stability of the recombinant analyte – standards (STD) and control (CTRL):

4 samples with recombinant analyte were subjected to 3 freeze/thaw cycles. After each cycle aliquots were drawn. All aliquots of different F/T cycles were assayed in one test run.

Data of OD values and recovery of reconstituted STDs and CTRL which were a) freshly reconstituted, b) stressed by 1 freeze/thaw cycle, c) stressed by 2 freeze/thaw cycles and d) stressed by 3 freeze/thaw cycles:

No. of F/T cycles	freshly reconst	1	2	3	fresh vs 1xF/T	fresh vs 2xF/T	fresh vs 3xF/T
Sample ID	OD				R(%)	R(%)	R(%)
STD3	0.346	0.337	0.363	0.344	97	105	99
STD4	1.173	1.107	1.114	1.148	94	95	98
STD5	3.392	3.307	3.400	3.335	98	100	98
CTRL	0.780	0.716	0.762	0.785	92	98	101
Mean (%)					95	99	99

The recovery of recombinant analyte stressed by three freeze/thaw cycles is 98% to 101%.

SAMPLE CHARACTERISTICS

Sample stability:

- NT-proBNP is stable in whole blood for several hours at room temperature. Nevertheless we recommend separating serum by centrifugation as soon as possible, e.g. 20 min at 2,000 x g, preferably at 4°C (2-8°C).
- Serum can be stored at 4°C (2-8°C) up to two days.
- For long term storage, aliquot the acquired serum samples and store at -25°C or lower.
- Samples can be subjected to 5 freeze-thaw cycles without any loss of immune reactivity.
- Serum samples can be stored for at least 2 years at -80°C.

Validation

The assay is fully validated according to ICH Q2 (R1), Ref. [1].

References

[1] CPMP/ICH/381/95 - ICH Topic Q2 (R1) „Validation of Analytical Procedures: Text and Methodology“ including:

ICH Q2A “Text on Validation of Analytical Procedures”

ICH Q2B “Validation of Analytical Procedures: Methodology”

Available on our homepage

Enzyme immunoassay for the quantitative determination of NT-proBNP (1-76) in human serum.

Package Insert, MSDS, Information Folder, and References:

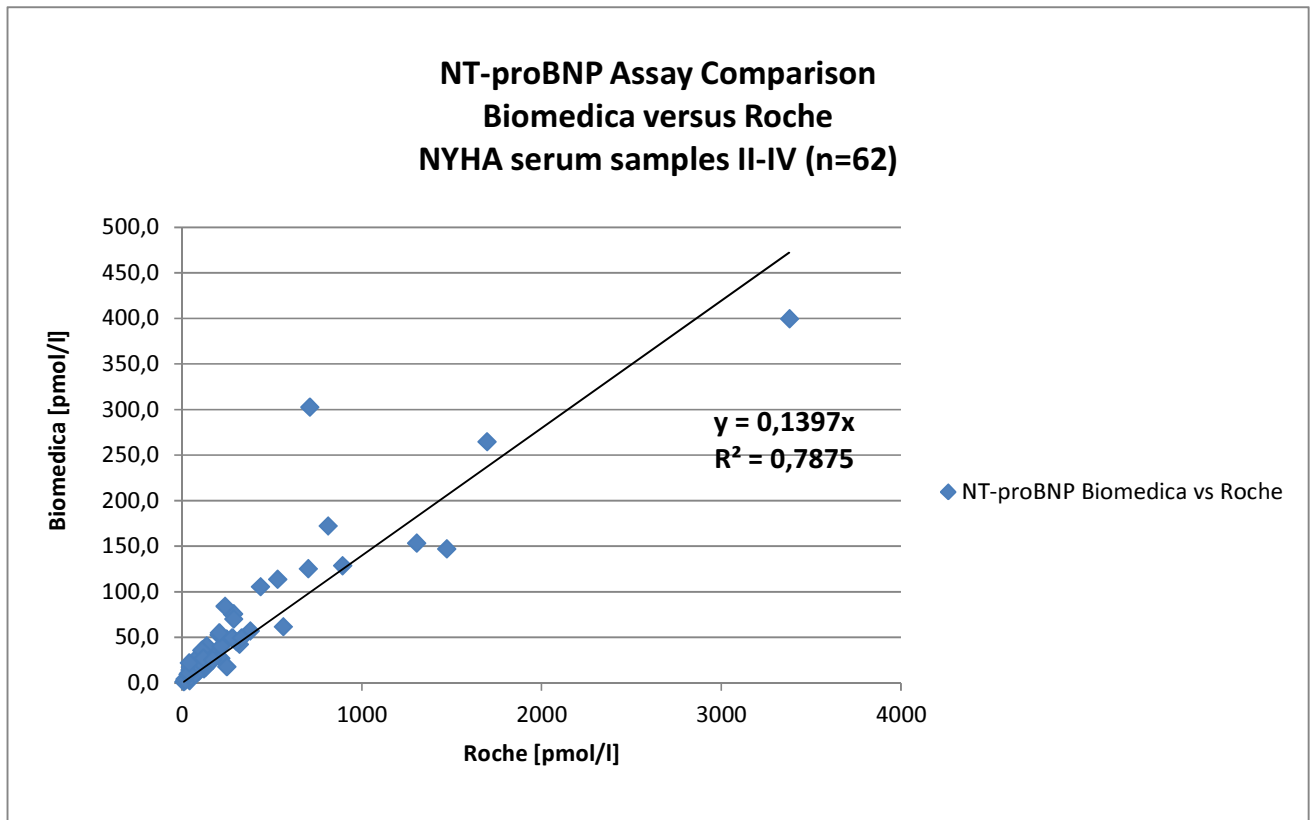
<http://www.bmgrp.com/products/cardiovascular/nt-probnp-elisa/>

NT-proBNP ASSAY COMPARISON

**Comparison between NT-proBNP Assays:
Biomedica SK-1204 versus Roche Elecsys 2nd generation Assay**

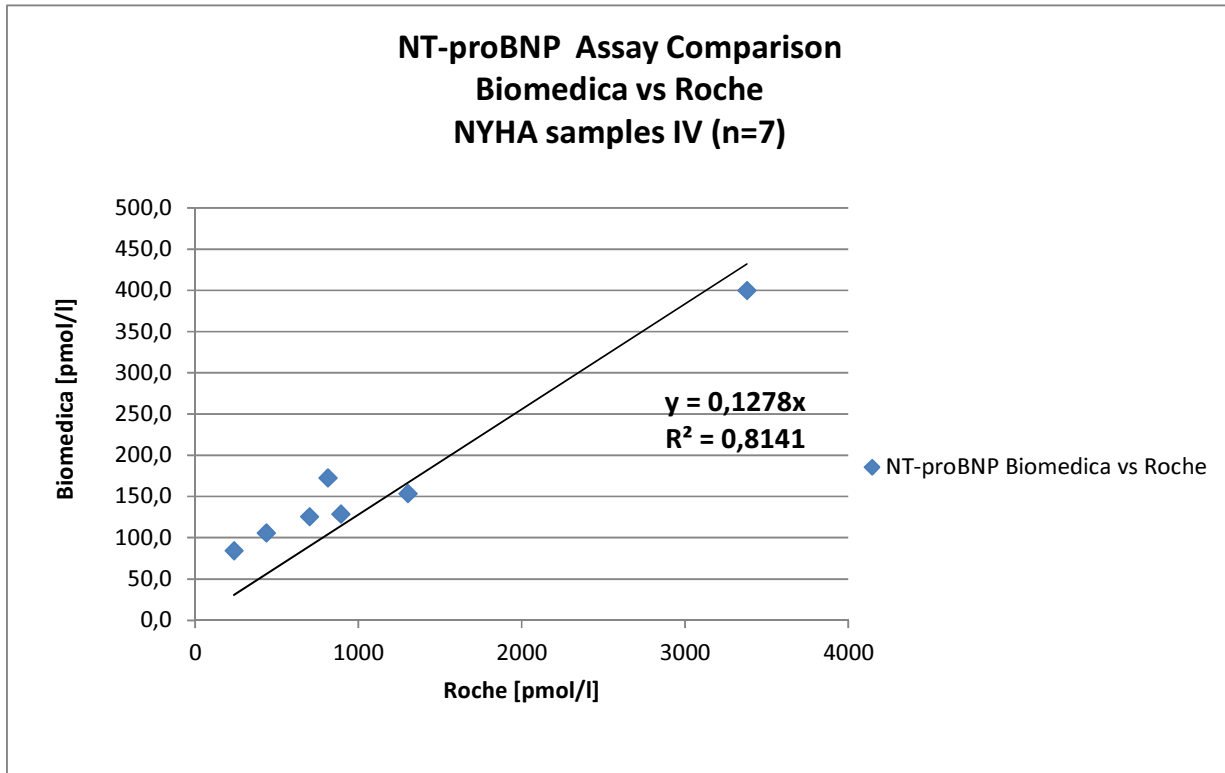
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n	62
mean	54.0 pmol/l
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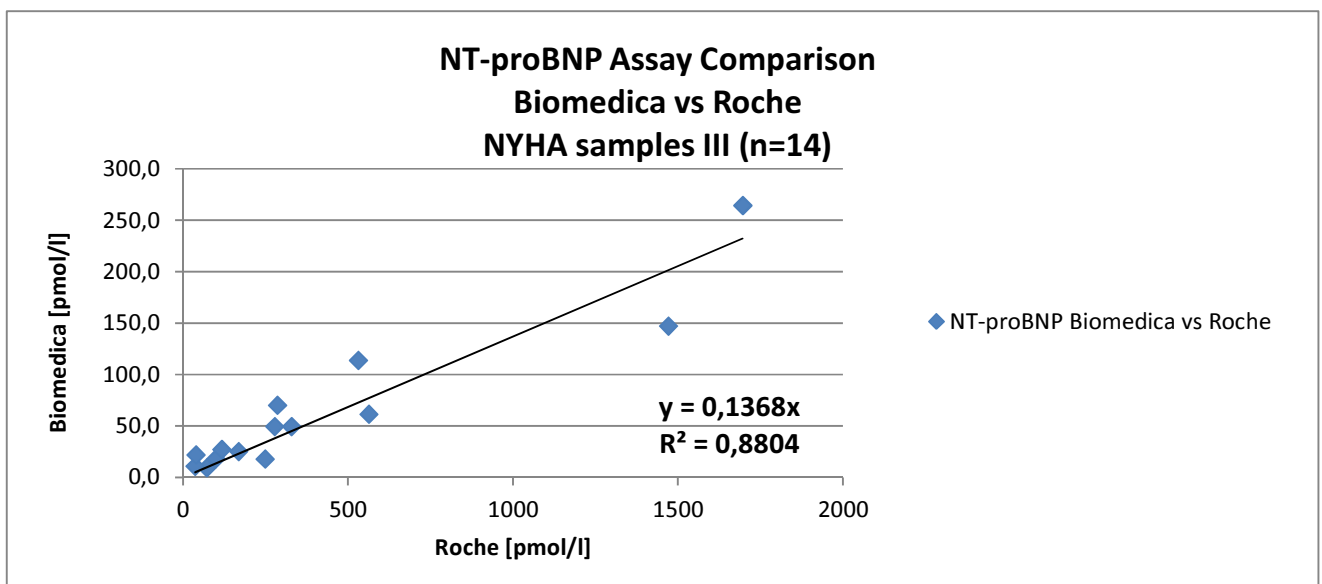
Data on serum values of a heart failure panel NYHA classification IV measured with the Biomedica NT-proBNP ELISA (#SK-1204)

n	7
mean	166.8 pmol/l
max	399.3 pmol/l
min	84.1 pmol/l



Data on serum values of a heart failure panel NYHA classification III measured with the Biomedica NT-proBNP ELISA (#SK-1204)

n	14
mean	63.2 pmol/l
max	264.3 pmol/l
min	9.2 pmol/l



Data on serum values of a heart failure panel NYHA classification II measured with the Biomedica NT-proBNP ELISA (#SK-1204)

n	41
mean	25,5 pmol/l
max	75,6 pmol/l
min	1,1 pmol/l

