NT-proCNP ELISA

(2nd generation)

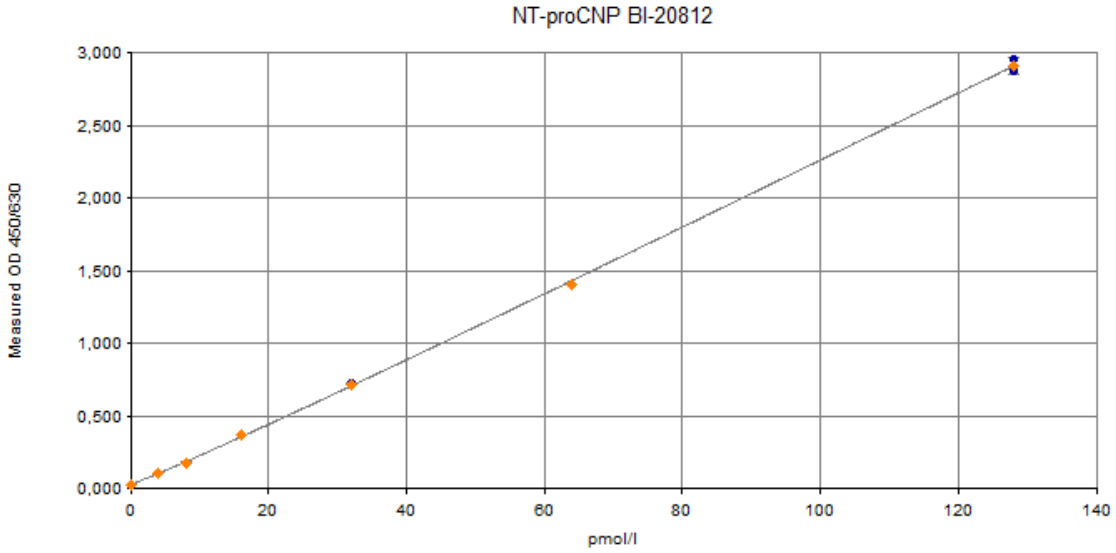
for the quantitative determination of human NT-proCNP
in serum, EDTA plasma, citrate plasma and heparin plasma
Cat. No. BI-20812 . 12 x 8 tests

Content

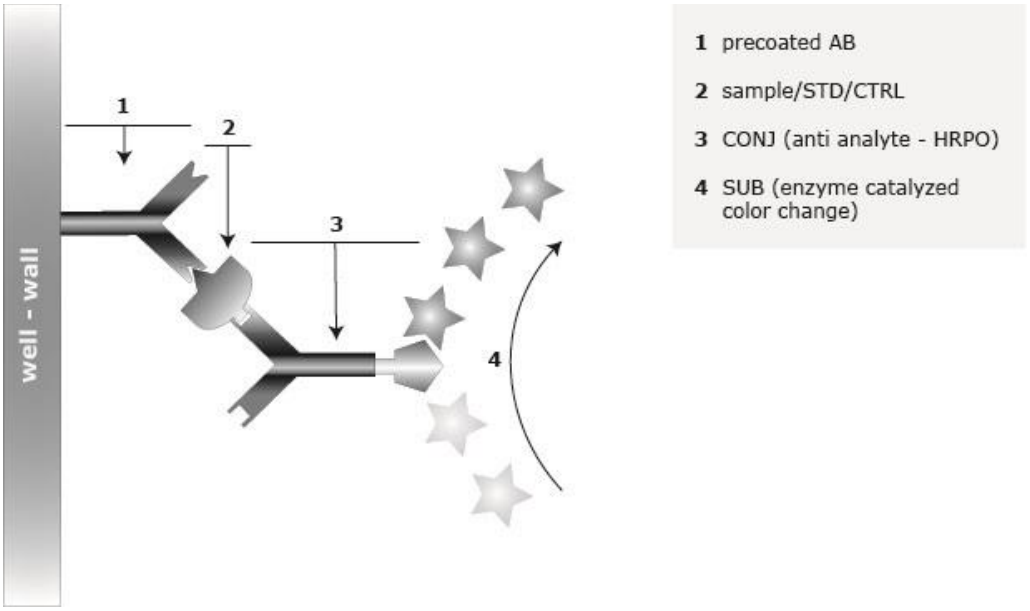
ASSAY CHARACTERISTICS Summary

Method	Sandwich ELISA, HRP/TMB, 12x8-well strips		
Sample type	Serum, EDTA plasma, citrate plasma, and heparin plasma Protocol for urine, cell culture supernatants and non-human species available		
Standard range	0 to 128 pmol/l (7 standards and 2 controls in a human serum matrix. Standards: 0/4/8/16/32/64/128 pmol/l)		
Conversion factor	1 pg/ml = 0.201 pmol/l (MW: 4.985 kDa) 1 pmol/l = 4.985 pg/ml		
Sample volume	20 µl / well		
Incubation time, temp.	20 min / 3 h / 30 min, room temperature		
Sensitivity	LOD: (0 pmol/l + 3 SD): 0.7 pmol/l; LLOQ: 0.5 pmol/l		
Specificity	This assay recognizes endogenous and synthetic human NT-proCNP		
Precision	Intra-assay (n=5) ≤ 6% Inter-assay (n=8) ≤ 7%		
Spike/Recovery	Average % recovery spiked with 64 pmol/l	Serum (n=6): 101 EDTA plasma (n=6): 99 Citrate plasma (n=2): 100 Heparin plasma (n=2): 93	
Dilution linearity of endogenous NT-proCNP	Average % of expected of dilution:	1+1	1+3
	Serum (n=6):	99	98
	EDTA plasma (n=6):	103	98
	Heparin plasma (n=2):	100	100
	Citrate plasma (n=2):	96	92
Values of apparently healthy individuals	Median serum (n=32) = 14.5 pmol/l Median EDTA plasma (n=33) = 15 pmol/l Median heparin plasma (n=18) = 13.5 pmol/l Median citrate plasma (n=18) = 12 pmol/l Each laboratory should establish its own reference range for the samples under investigation. Do not change sample type during the study.		

TYPICAL STANDARD CURVE



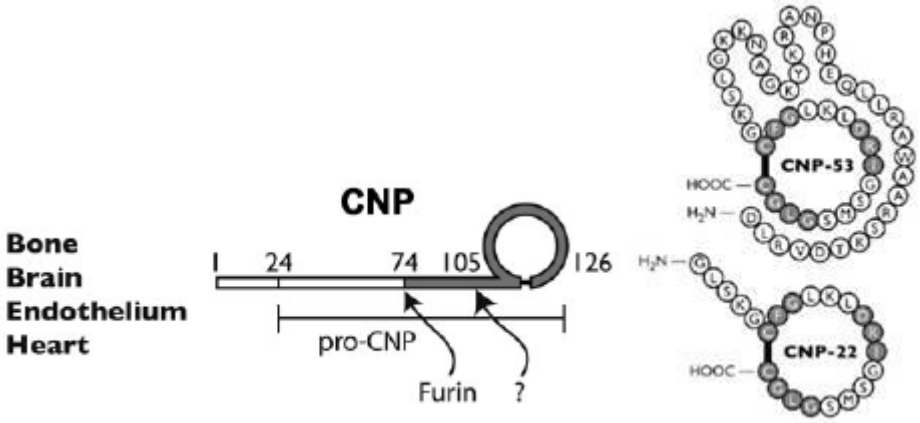
PRINCIPLE OF THE ASSAY



CAB Coating Antibody: polyclonal goat antibody (AA 53-73 of Uniprot ID # P23582)
DAB Detection Antibody: polyclonal goat antibody (AA 24-42 of Uniprot ID # P23582)
AG Antigen: synthetic human propeptide of CNP (AA 24-73 of Uniprot ID # P23582)

INFORMATION on the ANALYTE

The 103–amino acid propeptide is cleaved either between residues 50 and 51 or 81 and 82 to produce one of two biologically active peptides, carboxy-terminal proCNP (51–103) or carboxy-terminal proCNP (82–103), respectively, and an amino-terminal congener, N-terminal proCNP (1).

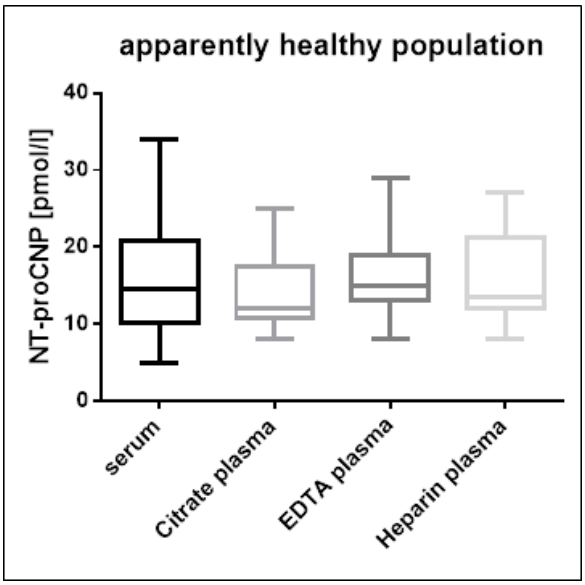


From: Natriuretic Peptides, Their Receptors, and Cyclic Guanosine Monophosphate-Dependent Signaling Functions. *Potter LR et al., 2006; Endocrine Reviews 27(1):47–72. doi: 10.1210/er.2005-0014*

SAMPLE VALUES

NT-proCNP levels in an apparently healthy cohort

	NT-proCNP [pmol/l]			
	Serum (n=32)	**EDTA plasma (n=33)**	**Heparin plasma (n=18)**	**Citrate plasma (n=18)**
Mean	15.78	16.18	15.83	14.06
Median	**14.5**	**15**	**13.5**	**12**
Percentile 95%	34	28.3	27	25
Percentile 5%	5.65	9.4	8	8
Min	5	8	8	8
Max	34	29	27	25



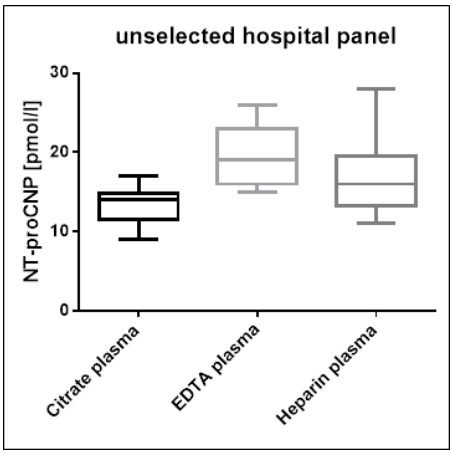
It is recommended to establish the normal range for each laboratory.

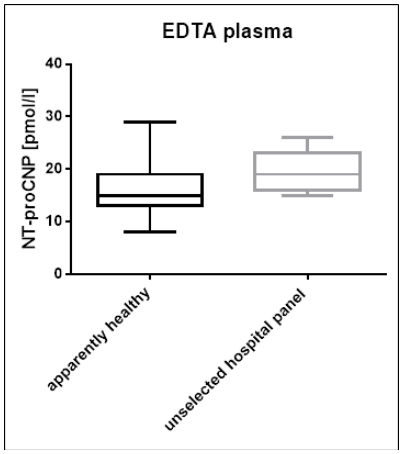
NT-proCNP levels in unselected hospital panels

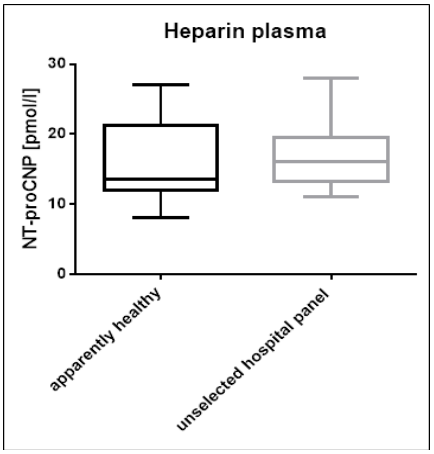
	NT-proCNP [pmol/l]		
	Citrate plasma (n=8)	**EDTA plasma (n=8)**	**Heparin plasma (n=8)**
Mean	13.38	19.38	17
Median	**14**	**19**	**16**
Percentile 95%	17	26	28
Percentile 5%	9	15	11
Min	9	15	11
Max	17	26	28

EDTA plasma	NT-proCNP [pmol/l]	
	App. healthy (n=33)	**Hospital cohort (n=8, unselected)**
Mean	16.18	19.38
Median	**15**	**19**
Percentile 95%	28.3	26
Percentile 5%	9.4	15
Min	8	15
Max	29	26

Heparin plasma	NT-proCNP [pmol/l]	
	App. healthy (n=18)	**Hospital cohort (n=8, unselected)**
Mean	15.83	17
Median	**13.5**	**16**
Percentile 95%	27	28
Percentile 5%	8	11
Min	8	11
Max	27	28







MATRIX COMPARISON

Comparison of NT-proCNP serum and plasma sample values from apparently healthy individuals

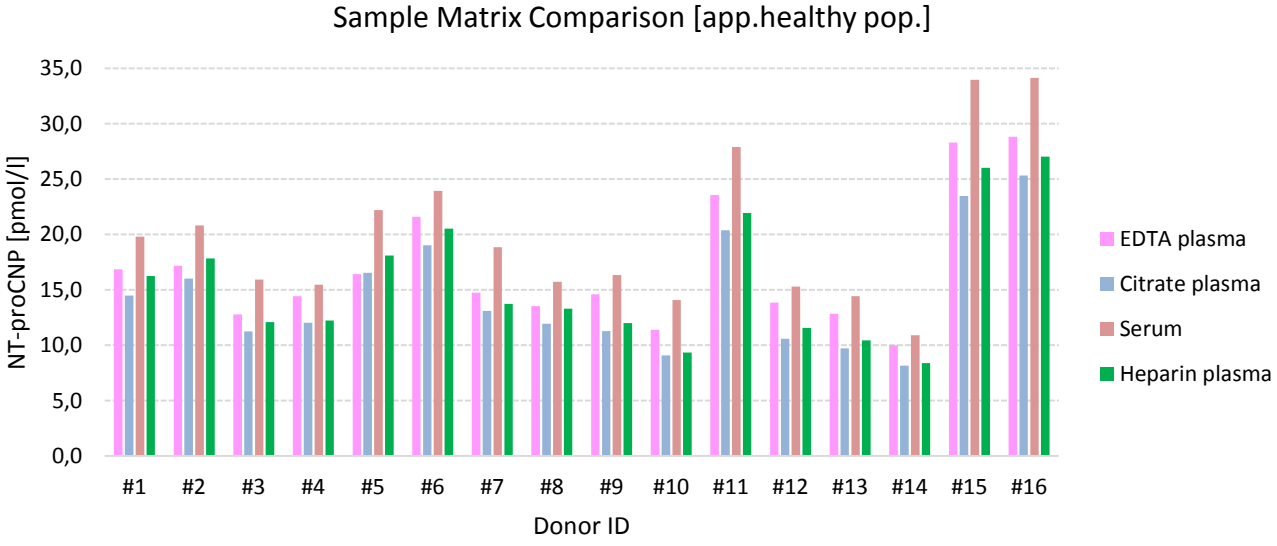
Comparison of human NT-proCNP sample concentrations between serum, EDTA plasma, and citrate plasma from an apparently healthy cohort (n=16).

Human NT-proCNP was measured in four matrices from sixteen individual donors.

	NT-proCNP [pmol/l]			
Donor ID	**EDTA plasma**	**Citrate plasma**	**Heparin plasma**	**Serum***
#1	17	14	16	20
#2	17	16	18	21
#3	13	11	12	16
#4	14	12	12	15
#5	16	17	18	22
#6	22	19	21	24
#7	15	13	14	19
#8	14	12	13	16
#9	15	11	12	16
#10	11	9	9	14
#11	24	20	22	28
#12	14	11	12	15
#13	13	10	10	14
#14	10	8	8	11
#15	28	23	26	34
#16	29	25	27	34

*Measured values of human NT-proCNP in serum are higher compared to plasma in an apparently healthy cohort (n=16).
It has been shown that sample values for serum and plasma can differ for the measurement of chemistry analytes (2).

Graph showing NT-proCNP levels dependence of sample concentrations on sample matrix



ASSAY PERFORMANCE CHARACTERISTICS

RECOVERY

Summary of data showing mean recovery of NT-proCNP:

Matrix	+12.8 pmol/l		+64 pmol/l	
	Mean	Range	Mean	Range
Serum (n=6)	102%	91-115%	101%	96-105%
EDTA plasma (n=6)	99%	80-116%	99%	94-101%
Citrate plasma (n=2)	100%	94-106%	100%	97-103%
Heparin plasma (n=2)	92%	90-94%	93%	92-95%

Experiments:
Recovery of spiked samples was tested by adding 2 concentrations synthetic NT-proCNP (12.8 + 64 pmol/l) to different human sample matrices.

Data showing spike/recovery of human **serum** samples:

Sample ID	Spike NT-proCNP [pmol/l]			S/R [%]	
	0	12.8	64	12.8	64
#S1	32.4	42.3	83.6	103	105
#S2	34.4	42.6	81.1	91	100
#S3	20.5	30.7	74.8	95	101
#S4	24.0	34.4	73.8	100	96
#S5	28.5	39.6	79.9	109	103
#S6	25.5	37.7	78.6	115	103
			Mean R [%]	**102**	**101**

Data showing spike/recovery of human **EDTA plasma** samples:

Sample ID	Spike NT-proCNP [pmol/l]			S/R [%]	
	0	12.8	64	12.8	64
#E1	29.0	36.3	78.7	80	100
#E2	31.5	43.1	78.9	116	99
#E3	25.3	35.5	77.2	99	101
#E4	28.4	38.7	78.2	103	100
#E5	27.7	38.0	73.9	102	94
#E6	21.3	30.9	74.4	92	100
			Mean R [%]	**99**	**99**

Data showing spike/recovery of human **heparin plasma** samples:

Sample ID	Spike NT-proCNP [pmol/l]			S/R [%]	
	0	12.8	64	12.8	64
#H1	28	37	75	90	95
#H2	30	39	74	94	92
			Mean R [%]	**92**	**93**

Data showing spike/recovery of human **citrate plasma** samples:

Sample ID	Spike NT-proCNP [pmol/l]			S/R [%]	
	0	12.8	64	12.8	64
#C1	24	35	78	106	103
#C2	29	38	77	94	97
			Mean R [%]	100	100

LINEARITY

Dilution linearity of samples containing endogenous NT-proCNP

Matrix	Recovery of dilution steps [%]			
	1+1		1+3	
	Mean	Range	Mean	Range
Serum (n=6)	99	96-102	98	93-119
EDTA plasma (n=6)	103	98-110	98	92-104
Heparin plasma (n=2)	100	98-103	100	97-102
Citrate plasma (n=2)	96	94-98	92	86-98

Dilution linearity of samples containing synthetic NT-proCNP

Matrix	Recovery of dilution steps [%]							
	1+1		1+3		1+7		1+15	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Serum (n=6)	93	89-97	89	78-97	86	78-100	99	89-118
EDTA plasma (n=6)	99	95-102	95	89-102	93	84-101	97	85-111
Heparin plasma (n=2)	102	94-111	100	94-105	92	84-99	93	90-96
Citrate plasma (n=2)	95	94-97	93	89-96	87	84-90	99	98-100

Experiment:

Dilution linearity was assessed by serially diluting samples containing endogenous NT-proCNP with ASYBUF (assay buffer supplied in the kit).

Data showing the dilution of endogenous NT-proCNP in **serum** samples:

Sample ID	NT-proCNP [pmol/l]			R [%]	
	ref	1+1	1+3	1+1	1+3
#S1	32.4	15.6	7.6	96	94
#S2	34.4	17.2	8.0	100	93
#S3	20.5	10.1	4.9	99	94
#S4	24.0	11.5	5.6	96	94
#S5	28.5	14.1	6.8	99	96
#S6	25.5	13.0	7.6	102	119
			Mean R [%]	99	98

Data showing the dilution of endogenous NT-proCNP in **EDTA plasma** samples:

Sample ID	NT-proCNP [pmol/l]			R [%]	
	ref	1+1	1+3	1+1	1+3
#E1	29.0	14.3	6.9	99	95
#E2	31.5	17.4	8.2	110	104
#E3	25.3	12.8	6.1	101	97
#E4	28.4	13.9	6.5	98	92
#E5	27.7	15.2	7.1	110	102
#E6	21.3	10.7	5.2	101	98
			Mean R [%]	**103**	**98**

Data showing the dilution of endogenous NT-proCNP in **citrate plasma** samples:

Sample ID	NT-proCNP [pmol/l]			R [%]	
	ref	1+1	1+3	1+1	1+3
#C1	24.1	11.4	6.9	94	86
#C2	28.8	14.1	5.2	98	98
			Mean R [%]	**96**	**92**

Data showing the dilution of endogenous NT-proCNP in **heparin plasma** samples:

Sample ID	NT-proCNP [pmol/l]			R [%]	
	ref	1+1	1+3	1+1	1+3
#H1	28.1	13.7	6.8	98	97
#H2	29.7	15.,3	7.6	103	102
			Mean R [%]	**100**	**100**

Recommendations for sample dilution
High measuring samples outside of the calibration range of the curve should be **diluted with ASYBUF** (assay buffer, supplied in the kit).

PRECISION

Intra-assay precision & Inter-assay precision

Intra-assay (n=5) ≤ 6%, Inter-assay (n=8) ≤ 7%

Intra-assay: 2 samples of known concentrations were tested 5 times within 1 kit lot by 1 operator.
Inter-assay: 2 samples of known concentrations were tested 8 times within 2 different kit lots by 2 different operators.

Intra-assay (n=5)	Sample 1	Sample 2
Mean (pmol/l)	7.9	65.3
SD (pmol/l)	0.47	1.25
CV (%)	6	2

Inter-assay (n=8)	Sample 1	Sample 2
Mean (pmol/l)	8.2	64.1
SD (pmol/l)	0.54	1.42
CV (%)	7	2

SENSITIVITY

Limit of detection (LOD)

The LOD is defined as the mean value of the back calculated concentration plus three times the standard deviation. The LOD of the NT-proCNP ELISA is **0.7 pmol/l**.

Lower limit of quantification (LLOQ)

The lower limit of quantification is defined as the lowest concentration where the following two criteria are met: 1) back fit of the calibration standards shall be within 75 – 125% and 2) precision shall be ≤ 25% (acc. to ICH [Ref. 1]).
The LLOQ for the NT-proCNP ELISA is **0.5 pmol/l**.

SAMPLE STABILITY

Sample preparation

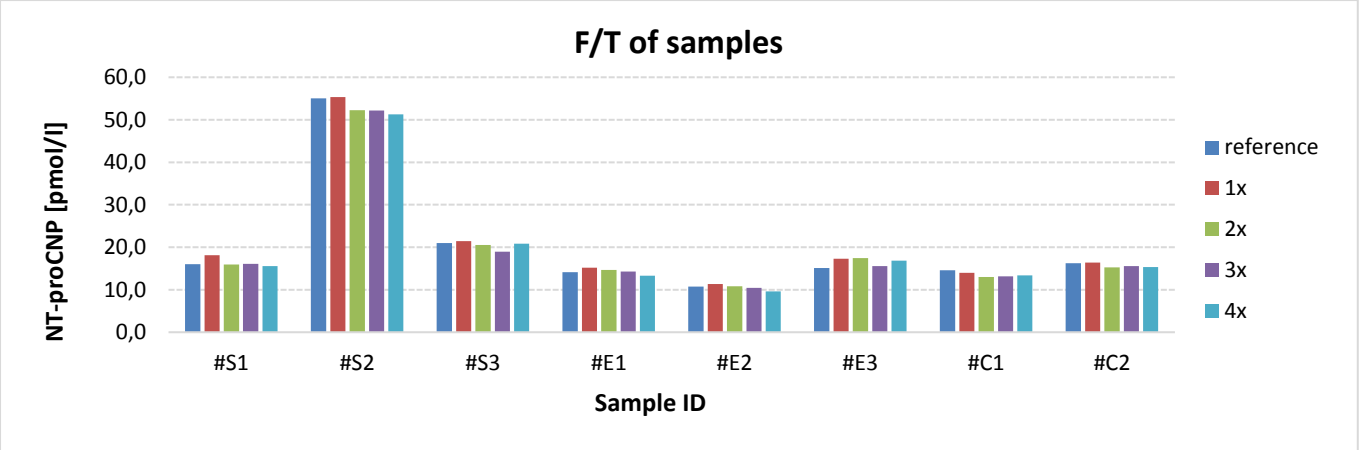
Collect venous blood samples by using standardized blood collection tubes for serum or plasma. Perform serum and plasma separation by centrifugation according to supplier's instructions of the blood collection devices as soon as possible.
The acquired serum or plasma samples should be measured as soon as possible. For longer storage aliquot samples and store at -25°C or lower.

Freeze-thaw of serum samples containing endogenous NT-proCNP

A set of samples (3 sera, 3 EDTA plasma, 2 citrate plasma) was aliquoted and freeze-thaw stressed. The reference samples are freeze thawed once. Samples can undergo 4 freeze-thaw cycles. The mean recovery of sample concentrations stressed by 4 F/T cycles is 96%. According to our data, samples can undergo at least 4 freeze-thaw cycles.

NT-proCNP concentrations of samples after freeze-thaw cycles:

	NT-proCNP [pmol/l]				R [%]
Sample ID	**reference**	**2x**	**3x**	**4x**	**4 F/T vs ref**
#S1	16.0	15.9	16.1	15.6	98
#S2	55.1	52.3	52.2	51.3	93
#S3	21.0	20.5	18.9	20.8	99
#E1	14.2	14.6	14.3	13.3	94
#E2	10.7	10.8	10.4	9.6	90
#E3	15.1	17.4	15.6	16.8	112
#C1	14.6	13.0	13.1	13.4	92
#C2	16.2	15.3	15.6	15.4	95
				Mean R [%]	**96**



SPECIFICITY
This assay recognizes endogenous (natural) and synthetic human NT-proCNP.

CALIBRATION
This immunoassay is calibrated against synthetic human propeptide of CNP (AA 24-73 of Uniprot ID# P23582) (= NT-proCNP 1-50).

COMPARISON of NT-proCNP ELISA # BI-20812 (2nd generation) vs # BI-20872

The two NT-proCNP assays show a poor correlation due to the use of different reagents (e.g standard material, highly purified antibodies, and buffer solutions).

VALIDATION GUIDELINES

The assay is fully validated for human serum and plasma samples according to ICH Q2 (R1) (3).

LITERATURE
1) Amino-Terminal Pro–C-Type Natriuretic Peptide in Heart Failure. *Wright SP et al., Hypertension, 2004; 43:94-100.*
2) Comparison of Serum and Heparinized Plasma Samples for Measurement of Chemistry Analytes. *Miles RR et al., Clin Chem, 2004; 50, 9: 1704-1705.*
3) 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 Website **www.bmgrp.com**
Instructions for Use (package insert)
Protocols for urine, cell culture supernatants and non-human samples
Material Safety Data Sheet

MEASUREMENT OF NT-proCNP in human URINE, CELL CULTURE SUPERNATANTS and in NON-HUMAN SAMPLES

The following experiments have been performed to test the use of the NT-proCNP assay (cat. no. BI-20812) in human urine, human cell culture supernatants, and in rat, mouse, and pig samples.

Note: the experiments performed for these samples did not undergo a full validation and are therefore merely a performance check.

1. MEASUREMENT of NT-proCNP in HUMAN URINE SAMPLES

Undiluted human urine samples from apparently healthy subjects and from patients with kidney disease were tested.

Summary:

Urine samples (n=48) were assayed with the Biomedica NT-proCNP ELISA (BI-20812) following the standard protocol **using undiluted urine.**

- Endogenous NT-proCNP was detectable in samples from kidney patients.
- Urine samples can be spiked – the average recovery of 8 human urine samples is 109%.
- If required, dilute urine samples 1+1 with assay buffer.
  Dilution linearity of samples containing high values of endogenous NT-proCNP (n=2) with assay buffer is 103%.
- Specificity was assessed by adding the coating antibody utilized in the NT-proCNP ELISA assay to urine samples containing elevated endogenous NT-proCNP levels (n=2). The samples showed a competition of 93%.

RECOVERY

Recovery was assessed by adding STD7 (final concentration 64 pmol/l) synthetic human NT-proCNP directly to eight different human urine samples (ratio 1+1).

Data showing spike/recovery of human urine samples:

	NT-proCNP c [pmol/l]		
Sample ID	**Reference**	**+ 64 pmol/l**	**S/R [%]**
#U1	1	68	106
#U2	59	111	128
#U3	1	67	105
#U4	1	67	104
#U5	0	63	98
#U6	80	116	118
#U7	0	64	100
#U8	1	70	109
		Mean R [%]	**109**

LINEARITY

Dilution linearity was assessed by diluting urine samples containing endogenous NT-proCNP with ASYBUF (assay buffer supplied in the kit).

Data showing the dilution of endogenous NT-proCNP in urine samples:

Sample ID	NT-proCNP [pmol/l]		Dil R [%]
	Reference	Dil 1+1	
#U1	69	33	95
#U2	92	51	111
		Mean R [%]	**103**

COMPETITION

Specificity was assessed by adding the coating antibody utilized in the NT-proCNP ELISA assay to urine samples containing elevated endogenous NT-proCNP levels.

Data showing the competition of the signal:

	NT-proCNP [pmol/l]		
Sample ID	**Reference**	**Competition**	**Comp R [%]**
#U1	69	6	91
#U2	92	4	96
		Mean R [%]	**93**

Comparison of panels from various donors

	NT-proCNP [pmol/l]		
	App. healthy (n=4)	**Kidney cohort I (n=24)**	**Kidney cohort II (n=20)**
Mean	1.25	9.5	0.35
Median	**1**	**1**	**0**
Percentile 95%	2	86.25	1.95
Percentile 5%	1	0	0
Min	1	0	0
Max	2	92	2

Suggested protocol for the measurement of human NT-proCNP in urine samples

Follow standard protocol as indicated in the package insert:

Pipette **20 µl** of **undiluted urine sample** directly into the well of the microtiter plate.

If required, dilute samples 1+1 with assay buffer (provided in the kit).

2. MEASUREMENT of NT-proCNP in CELL CULTURE SUPERNATANTS

Note: the experiments performed to measure NT-proCNP in cell culture supernatants are not a full validation but are merely a performance check.

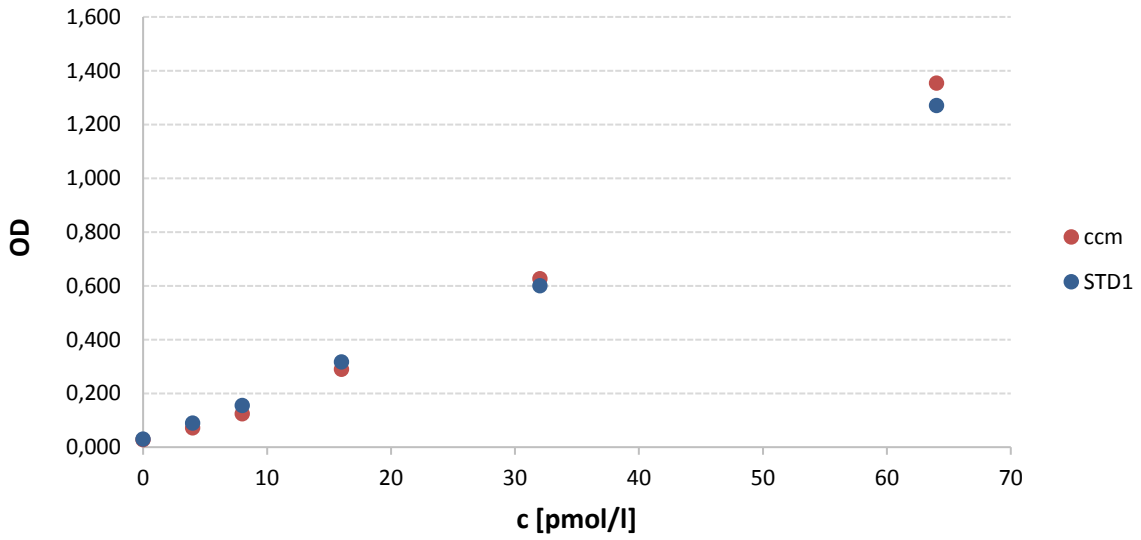
Cell culture medium (ccm: RPMI1640 containing 10% fetal calf serum) was tested undiluted and spiked with a final concentration of 64 pmol/l synthetic human NT-proCNP. The spiked solution was diluted 1+1, 1+3, 1+7 with the cell culture medium.

As a comparison, the spike recovery and dilution linearity of the standard matrix (=STD1) and the dilutions with assay buffer is shown.

OD values of spiked and diluted cell culture medium sample and standard matrix (STD1)

		OD					
Dil medium	**Sample ID**	**Reference**	**+ 64 pmol/l**	**1+1**	**1+3**	**1+7**	**1+15**
ccm	ccm	0.028	1.355	0.627	0.290	0.125	0.072
ASYBUF	STD1	0.031	1.271	0.601	0.318	0.156	0.090

Graph showing dilution of cell culture medium (ccm) and a comparison to the standard (STD1), both spiked with spiked with the same amount of synthetic NT-proCNP (64 pmol/l).



Suggested protocol for the measurement of human NT-proCNP in cell culture supernatants

Preparation of a cell culture medium based standard curve:

Reconstitute STD7 in 300 µl deionized water. Leave at room temperature (18-26°C) for 15 min and mix well prior to making dilutions.

Use polypropylene tubes.
For the preparation of the cell culture based standards *always* use the identical cell culture medium in which the samples are based on.

- Mark tubes e.g. CC STD6, CC STD 5 … CC STD1.
- Prepare a two-fold serial dilution to obtain STD6 to STD2.

e.g.:
Dispense 100 µl cell culture medium into vials labelled with CC STD6 to CC STD1.
Pipette 100 µl of STD 7 into tube marked as CC STD6. Mix thoroughly.
Transfer 100 µl of CC STD6 into vial marked as CC STD5. Mix thoroughly. Continue in the same fashion to obtain CC STD4 to CC STD2.

- Cell Culture Medium serves as the zero standard (=CC STD1, 0 pmol/l).

Attention: Concentrations defined for CTRL A and B are only valid for measuring NT-proCNP in human serum or plasma. The controls cannot be used for cell culture measurements.

3. MEASUREMENT OF NT-proCNP in NON-HUMAN SAMPLES

The sequence homology of NT-proCNP is very conserved among different species. We therefore assessed if the assay which is validated for human samples can be used in rat, mouse and pig samples. Other species types that share a high homology to human NT-proCNP can likely be measured with this assay (e.g. monkey, cats, dogs).

Note: the experiments performed for non-human species are not a full validation but are merely a performance check.

uniprot ID	species	sequence homology [%]
P23582	C-type natriuretic peptide Homo sapiens (Human)	100
H2QJL6	PANTR - Uncharacterized protein Pan troglodytes (Chimpanzee)	98
H2P8X1	PONAB - Uncharacterized protein - Pongo abelii (Sumatran Orangutan)	98
M3WH43	FELCA - Uncharacterized protein - Felis catus (Cat)	96
E2R4X2	CANLF - Uncharacterized protein - Canis lupus family	96
P18104	PIG - C-type natriuretic peptide Sus scrofa (Pig)	94
P55207	RAT - C-type natriuretic peptide - Rattus norvegicus (Rat)	92
Q9D288	MOUSE - Putative uncharacterized protein Mus musculus (Mouse)	86
Q544K5	MOUSE - C-type natriuretic peptide (Mus musculus)	86

Q61839	MOUSE - C-type natriuretic peptide Mus musculus (Mouse)	86
A0A1L1WKH8	RAT - Natriuretic peptide C - Rattus norvegicu	93

For more information on the sequence homology between various species for the natriutetic peptides, NT-proCNP, NT-proANP, and NT-proBNP please visit our website www.bmgrp.com, Sequence homology xNPs.

Suggested protocol for the measurement of NT-proCNP in non-human samples (e.g. rat or pig samples)

Follow standard protocol as indicated in the package insert:

Pipette **20 µl** of **undiluted sample** directly into the well of the microtiter plate.

If required, dilute samples 1+1 with assay buffer (provided in the kit).

A. Measurement of NT-proCNP in rat samples - performance check

Rat NT-proCNP shares a 92% homology to human NT-proCNP.
According to our data, rat serum samples (n=8) showed a recovery of 97% and a linearity of 87%. The samples tested contained endogenous NT-proCNP concentrations between 9-38 pmol/ml. Competition of endogenous NT-proCNP concentrations from rat samples is 100%.

RECOVERY and LINEARITY

Eight undiluted rat serum samples were tested in the NT-proCNP ELISA (note: standards contain synthetic human NT-proCNP spiked in a human serum matrix).
For Linearity experiments: rat samples were diluted 1+1 with ASYBUF (supplied in the kit).
For Recovery experiments: STD7 was added to the rat serum samples in a ratio 1+1 (final concentration 64 pmol/l).

Calculation of rat sample concentrations, and spike recovery

	NT-proCNP [pmol/l]			**NT-proCNP**	
Sample ID	**Reference**	**dilution 1+1**	**dil lin R [%]**	**+ 64 pmol/l**	**S/R [%]**
#R1	16	8	95	68	94
#R2	32	13	83	76	95
#R3	31	11	72	76	95
#R4	10	5	101	68	99
#R5	26	11	84	78	101
#R6	38	15	79	81	97
#R7	23	10	85	74	97
#R8	9	4	93	66	97
		Mean R [%]	**87**	**Mean S/R [%]**	**97**

COMPETITION

Specificity was assessed by adding the coating antibody utilized in the human NT-proCNP ELISA assay to the rat serum samples.

Data showing the competition of the signal:

	NT-proCNP [pmol/l]		
Sample ID	**Reference**	**+ coating AB**	**R [%] comp.**
#R1	15	0	100
#R2	28	0	100
#R3	29	0	100
#R4	10	0	100
#R5	27	0	100
#R6	34	0	100
#R7	22	0	100
#R8	9	0	100
		Mean R [%]	**100**

B. Measurement of NT-proCNP in mouse samples - performance check

Mouse NT-proCNP shares an 86% homology to human NT-proCNP.
According to our data, mouse serum samples (n=8) showed an average recovery of 45% when spiked with STD7 in a ratio 1+1 (final concentration 64 pmol/l). The mouse samples tested did not contain endogenous NT-proCNP concentrations. Linearity was not tested.

C. Measurement of NT-proCNP in pig samples - performance check

Pig NT-proCNP shares a 94% homology to human NT-proCNP.
According to our data, pig serum samples (n=8) showed a recovery of 83%. The pig samples tested did not contain endogenous NT-proCNP concentrations. Linearity was not tested.

RECOVERY

Eight undiluted pig serum samples were tested in the NT-proCNP ELISA (note: standards contain synthetic human NT-proCNP spiked in a human serum matrix).
For Recovery experiments: STD7 was added to the pig serum samples in a ratio 1+1 (final concentration 64 pmol/l).

Calculation of pig sample concentrations, and spike recovery:

	NT-proCNP [pmol/l]		
Sample ID	**Reference**	**+ 64 pmol/l**	**S/R [%]**
#P1	0	58	91
#P2	0	57	89
#P3	0	55	85
#P4	0	55	86
#P5	0	52	81
#P6	0	51	80
#P7	0	48	75
#P8	0	50	78
		Mean R [%]	**83**

Date: December 2017