Total soluble Neuropilin-1 ELISA

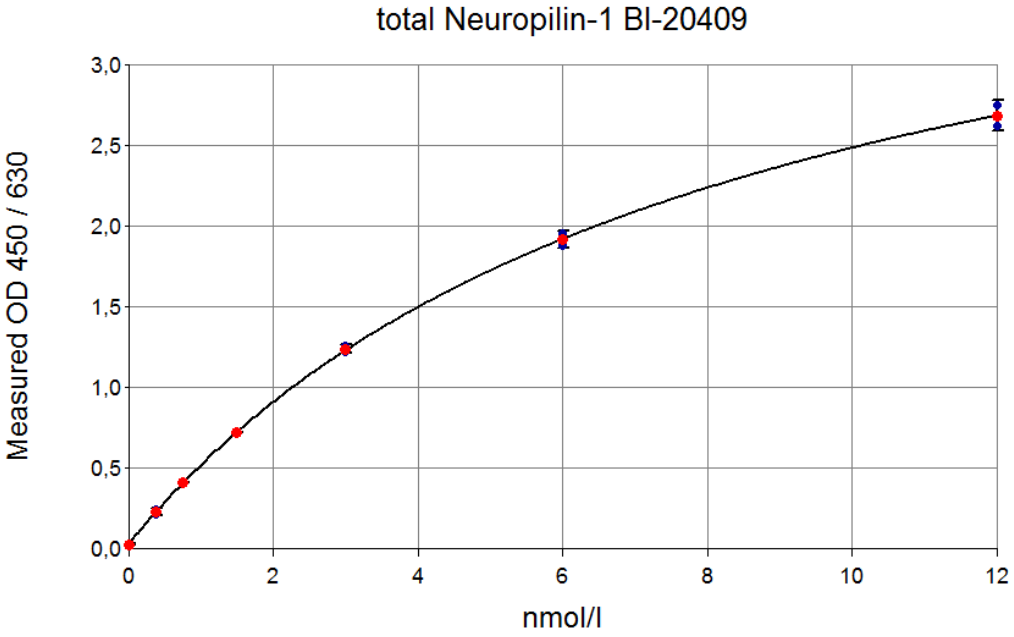
for the quantitative determination of human
soluble Neuropilin-1 in serum, EDTA plasma, heparin plasma, and citrate plasma
Cat. No. BI-20409. 12 x 8 tests

CONTENTS

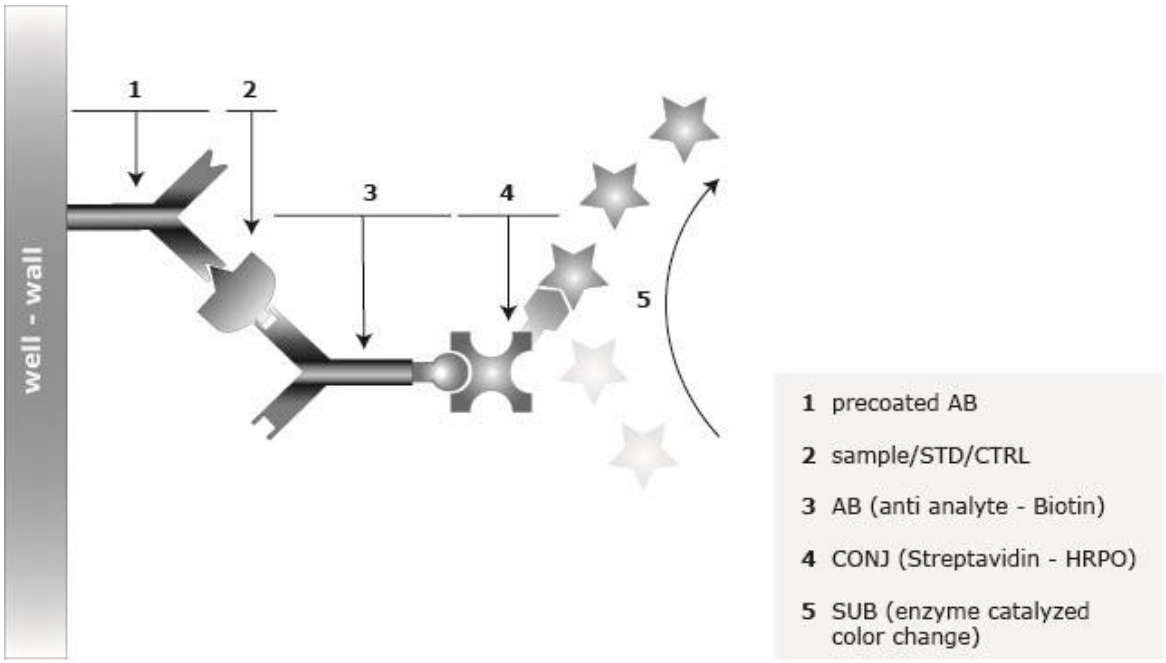
ASSAY CHARACTERISTICS Summary

Method	Sandwich ELISA, HRP/TMB, 12x8-well strips		
Sample type	Serum, EDTA plasma, heparin plasma, and citrate plasma		
Standard range	0 to 12 nmol/l (0 / 0.37 / 0.75 / 1.5 / 3 / 6 / 12 nmol/l) 7 standards and 2 controls in a serum matrix.		
Conversion factor	soluble Neuropilin-1: 1 ng/ml = 0.014 nmol/l; 1 nmol/l=69.7 ng/ml (MW: 69.7 kDa)		
Sample volume	10 µl / well		
Incubation time, temp.	30 min / 2 h / 1 h / 30 min, room temperature		
Sensitivity	LOD (0 pmol/l + 3 SD): 0.09 nmol/l; LLOQ: 0.09 nmol/l		
Specificity	The assay is optimized to detect soluble total Neuropilin-1 (NRP1) in human plasma and serum. This assay recognizes endogenous and recombinant human soluble Neuropilin-1 (isoform 2 and 3). The total soluble Neuropilin-1 ELISA utilizes a monoclonal anti-human Neuropilin-1 antibody that binds to a linear epitope close to the N-terminus in the CUB 1 domain of the Neuropilin-1 molecule. The polyclonal detection antibody binds to multiple linear epitopes, distributed over the entire Neuropilin-1 molecule. The human sequence homology between NRP1 and NRP2 is low. The amino acid sequence in the respective binding regions of the antibodies show no homology with NRP2. Thus a cross reactivity to NRP2 is not expected.		
Precision	Intra-assay (n=6) ≤ 11%, Inter-assay (n=12) ≤ 10%		
Spike/Recovery (recombinant 1.5 + 6 nmol/l Neuropilin-1)	**Average % recovery**	**1.5 nmol/l**	**6 nmol/l**
	Serum (n=6)	90	92
	EDTA plasma (n=6)	91	93
	Citrate plasma (n=1)	98	108
	Heparin plasma (n=1)	115	97
Dilution linearity of endogenous soluble Neuropilin-1	**Average % of expected of dilution:**	**1+1**	**1+3**
	Serum (n=6):	95	104
	EDTA plasma (n=6):	107	110
	Citrate plasma (n=1):	101	102
	Heparin plasma (n=1):	99	104
Values of apparently healthy individuals	Median serum (n=24) = 2.0 nmol/l Median EDTA plasma (n=24) = 1.7 nmol/l Median heparin plasma (n=24) = 1.9 nmol/l Median citrate plasma (n=24) = 1.7 nmol/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 anti-human Neuropilin-1 IgG
DAB Detection antibody: Monoclonal mouse antibody IgG, biotin labelled
AG Antigen: recombinant human Neuropilin-1 protein
(AA22-AA644 of Uniprot ID O14786-2, isoform 2)

INFORMATION on the ANALYTE

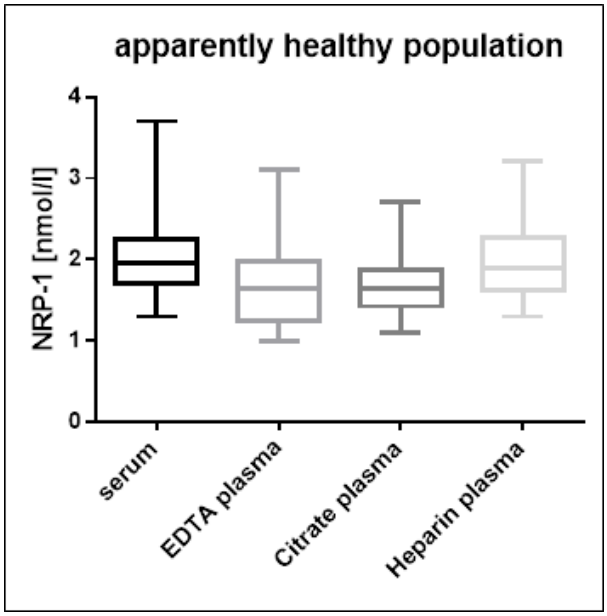
Neuropilin-1 (NRP1) is a single-pass transmembrane glycoprotein of 923 amino acids, composed of a large extracellular region, a short transmembrane domain and a short cytoplasmic tail https://www.uniprot.org/uniprot/O14786. Due to alternative splicing or shedding, the extracellular region can be released into circulation as soluble Neuropilin. NRP1 is an essential cell surface receptor functioning in many key biological processes including the cardiovascular, neuronal, and immune systems (1,2). Multiple ligands bind to the extracellular region of NRP1, like class III semaphorins which have a key role in axonal guidance, or members of the VEGF family of angiogenic cytokines. Ligand-binding to transmembrane NRP1, which has co-receptor function, leads to signaling via receptor proteins containing a PDZ domain. In contrast, ligand-binding to soluble Neuropilin-1 (sNRP1) has antagonistic properties by acting as decoy (1,3). NRP1 is expressed by a variety of cells and tissues. For instance, the transmembrane protein is expressed by neuronal cells, endothelial cells, vascular smooth muscle cells, cardiomyocytes, multiple tumor cell lines and neoplasms, osteoblasts, naïve T cells or platelets. Expression of soluble Neuropilin-1 is further described in a variety of non-endothelial cells, e.g. in liver hepatocytes and kidney distal and proximal tubules. NRP1 is implicated in a multitude of physiological and pathological settings, e.g. in axon guidance, vascularization, tumor growth or regeneration and repair (4-9). Neuropilin-1 is described to stimulate osteoblast differentiation, to act as potential biomarker for the prediction of heart failure outcome or to play a role in renal fibrogenesis (6, 10,11). As a co-receptor for VEGF, NRP1 is a potential target for cancer therapies (12). The Neuropilin-1 enzyme immunoassay is a four hour ELISA to quantify human total soluble Neuropilin-1 (sNRP1). The assay is validated for human serum and plasma samples (EDTA, citrate, heparin) (13) (see validation data: www.bmgrp.com). To remove potentially bound ligands, samples are pre-treated with guanidine hydrochloride before testing. Recombinant human soluble Neuropilin-1, isoform 2, is used as calibrator.

SAMPLE VALUES

soluble Neuropilin-1 values in an apparently healthy cohort

	Neuropilin-1 [nmol/l]			
	Serum (n=24)	**EDTA plasma (n=24)**	**Citrate plasma (n=24)**	**Heparin plasma (n=24)**
Mean	2.0	1.7	1.7	2.0
Median	**2.0**	**1.7**	**1.7**	**1.9**
5% Percentile	1.3	1.0	1.1	1.3
95% Percentile	3.4	3.0	2.6	3.1
Minimum	1.3	1.0	1.1	1.3
Maximum	3.7	3.1	2.7	3.2

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

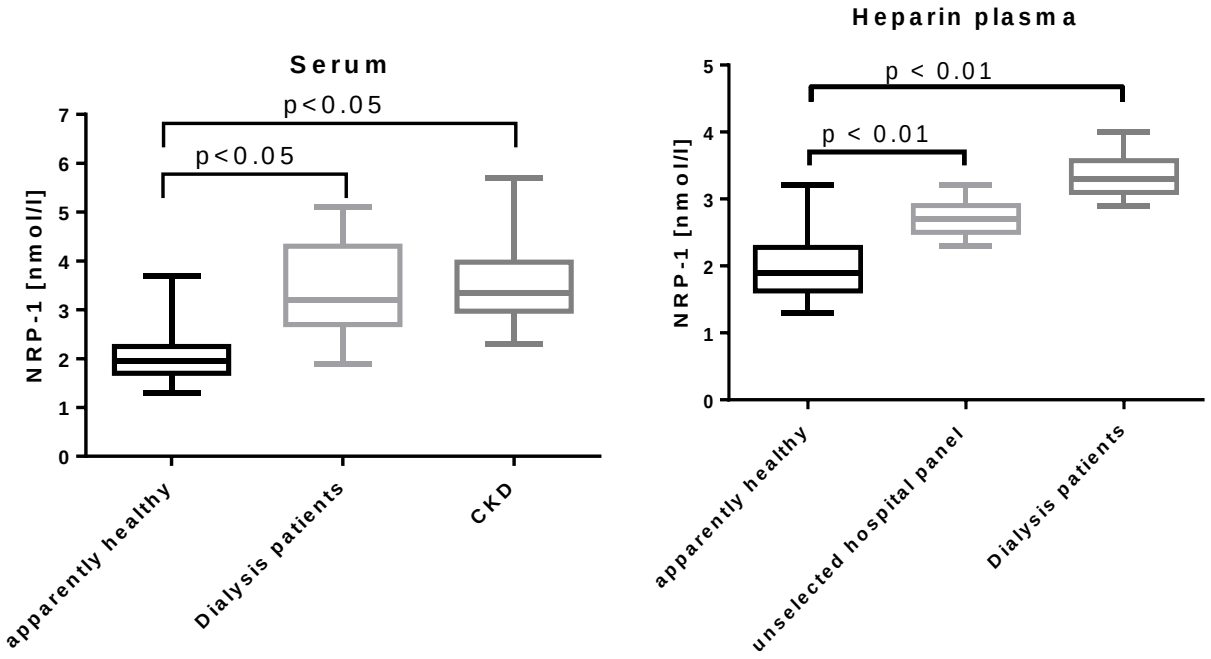


soluble Neuropilin-1 values in **serum** samples

	Neuropilin-1 [nmol/l]		
	Apparently healthy (n=24)	**Dialysis patients (n=15)**	**CKD (n=22)**
Mean	2.0	3.4	3.5
Median	**2.0**	**3.2**	**3.4**
5% Percentile	1.3	1.9	2.3
95% Percentile	3.4	5.1	5.6
Minimum	1.3	1.9	2.3
Maximum	3.7	5.1	5.7

soluble Neuropilin-1 values in **heparin plasma** samples

	Neuropilin-1 [nmol/l]		
	Apparently healthy (n=24)	**Unselected hospital panel (n=8)**	**Dialysis patients (n=16)**
Mean	2.0	2.7	3.3
Median	**1.9**	**2.7**	**3.3**
5% Percentile	1.3	2.3	2.9
95% Percentile	3.1	3.2	4
Minimum	1.3	2.3	2.9
Maximum	3.2	3.2	4



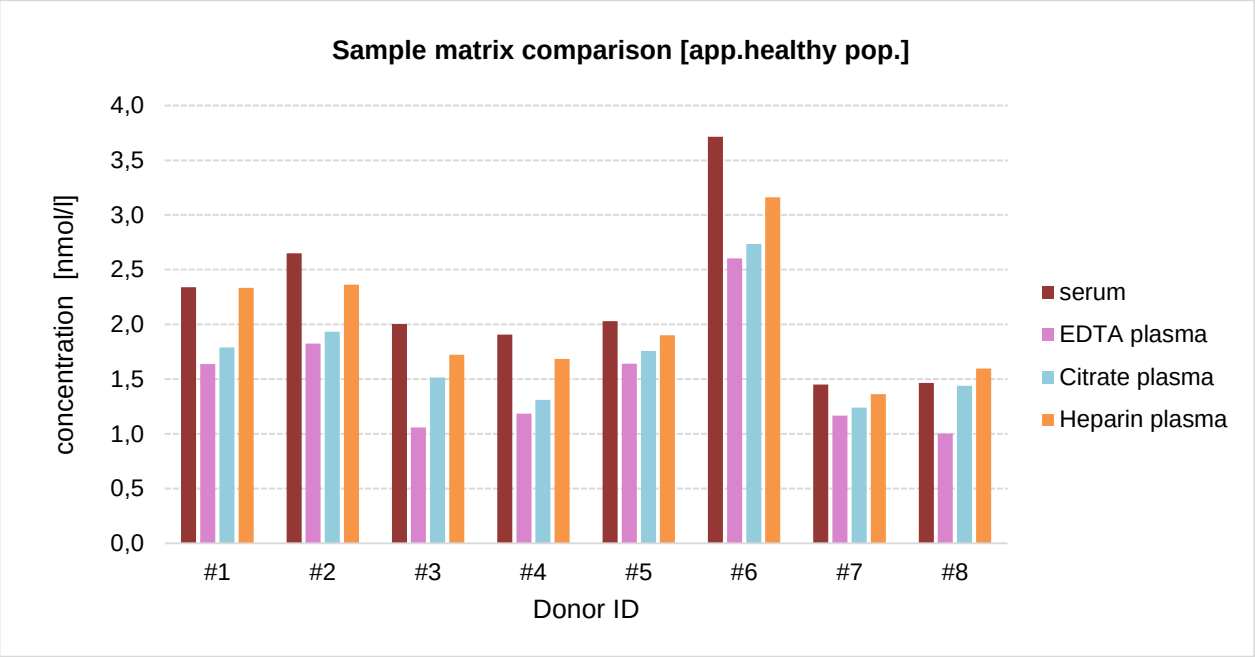
MATRIX COMPARISON

Comparison of Neuropilin-1 serum and plasma sample values from apparently healthy individuals

8 samples of apparently healthy individuals were prepared, each sample derived from one donor. Samples were assayed and the concentrations of the samples were compared.

	Neuropilin-1 [nmol/l]				CV [%]	
Donor ID	**Serum**	**EDTA plasma**	**Citrate plasma**	**Heparin plasma**	**all matrices**	**only plasma**
#1	2.3	1.6	1.8	2.3	16	16
#2	2.6	1.8	1.9	2.4	15	11
#3	2.0	1.1	1.5	1.7	22	19
#4	1.9	1.2	1.3	1.7	19	15
#5	2.0	1.6	1.8	1.9	8	6
#6	3.7	2.6	2.7	3.2	14	8
#7	1.5	1.2	1.2	1.4	8	6
#8	1.5	1.0	1.4	1.6	16	19
				Mean CV [%]	**15**	**13**

Graph showing soluble Neuropilin-1 levels in various sample matrices



ASSAY PERFORMANCE CHARACTERISTICS

RECOVERY

Summary of data showing mean recovery of soluble Neuropilin-1

Matrix	Mean S/R [%]	
	+ 1.5 nmol/l	+ 6 nmol/l
Serum (n=6)	90	92
EDTA plasma (n=6)	91	93
Citrate plasma (n=1)	98	108
Heparin plasma (n=1)	115	97

Experiments:
Recovery of spiked samples was tested by adding 2 concentrations of human recombinant Neuropilin-1 (1.5 + 6 nmol/l) to different human serum and plasma sample matrices.

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

	Spike Neuropilin-1 [nmol/l]			S/R [%]	
Sample ID	**0**	**1.5**	**6**	**1.5**	**6**
#S1	1.5	2.1	6.1	91	90
#S2	2.0	2.4	6.8	94	97
#S3	2.6	3.0	7.8	113	109
#S4	1.6	2.1	6.3	85	92
#S5	1.8	2.2	5.8	84	81
#S6	2.6	2.4	6.3	75	84
			Mean R [%]	**90**	**92**

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

	Spike Neuropilin-1 [nmol/l]			S/R [%]	
Sample ID	**0**	**1.5**	**6**	**1.5**	**6**
#E1	1.4	2.1	6.6	93	98
#E2	1.8	2.4	7.1	98	103
#E3	2.1	2.6	7.6	107	109
#E4	1.5	2.2	5.7	95	82
#E5	1.6	2.1	5.7	90	83
#E6	2.2	2.4	6.2	88	85
			Mean R [%]	**95**	**93**

Data showing spike/recovery of human **citrate plasma** sample

	Spike Neuropilin-1 [nmol/l]			S/R [%]	
Sample ID	**0**	**1.5**	**6**	**1.5**	**6**
#C1	1.9	2.4	7.4	98	108

Data showing spike/recovery of human **heparin plasma** sample

	Spike Neuropilin-1 [nmol/l]			S/R [%]	
Sample ID	**0**	**1.5**	**6**	**1.5**	**6**
#H1	2.2	2.8	6.9	115	97

LINEARITY

Dilution linearity of samples containing endogenous soluble Neuropilin-1

Matrix	Mean R of dilution steps [%]		
	1+1	**1+3**	**1+7**
Serum (n=6)	95	104	114
EDTA plasma (n=6)	107	110	115
Citrate plasma (n=1)	101	102	111
Heparin plasma (n=1)	99	104	110

- *We recommend diluting high measuring samples (outside of the calibration range) with STD1 (0 nmol/l, supplied in the kit).*

Experiment:
Dilution linearity was assessed by serially diluting samples containing endogenous soluble Neuropilin-1 with STD1.

Data showing the dilution of endogenous Neuropilin-1 in **serum** samples

Sample ID	Neuropilin-1 [nmol/l]				R [%]		
	ref	1+1	1+3	1+7	1+1	1+3	1+7
#S1	2.7	1.6	0.9	0.5	122	129	148
#S2	5.1	2.7	1.5	0.8	107	115	123
#S3	4.5	1.8	1.1	0.6	80	95	99
#S4	5.0	2.4	1.3	0.7	95	99	107
#S5	5.4	2.2	1.2	0.7	82	90	104
#S6	4.0	1.7	1.0	0.5	86	97	104
			Mean R [%]		**95**	**104**	**114**

Data showing the dilution of endogenous Neuropilin-1 in **EDTA plasma** samples

Sample ID	Neuropilin-1 [nmol/l]				R [%]		
	ref	1+1	1+3	1+7	1+1	1+3	1+7
#E1	2.8	1.6	0.9	0.5	117	127	137
#E2	2.7	1.3	0.7	0.4	98	105	106
#E3	3.4	1.9	0.9	0.5	110	111	114
#E4	3.2	1.7	0.9	0.4	108	114	111
#E5	3.3	1.8	0.9	0.5	111	107	117
#E6	3.9	2.0	0.9	0.5	101	97	105
			Mean R [%]		**107**	**110**	**115**

Data showing the dilution of endogenous Neuropilin-1 in **citrate plasma** samples

Sample ID	Neuropilin-1 [nmol/l]				R [%]		
	ref	1+1	1+3	1+7	1+1	1+3	1+7
#C1	2.7	1.4	0.7	0.4	101	102	111

Data showing the dilution of endogenous Neuropilin-1 in **heparin plasma** samples

Sample ID	Neuropilin-1 [nmol/l]				R [%]		
	ref	1+1	1+3	1+7	1+1	1+3	1+7
#H1	3.1	1.5	0.8	0.4	99	104	110

Dilution linearity of samples containing recombinant soluble Neuropilin-1

Matrix	Mean R of dilution steps [%]		
	1+1	1+3	1+7
Serum (n=3)	95	98	99
EDTA plasma (n=3)	84	96	94
Citrate plasma (n=1)	77	90	88
Heparin plasma (n=1)	95	104	104

Recommendations for sample dilution:

- *We recommend diluting high measuring samples (outside of the calibration range) with STD1 (0 nmol/l, supplied in the kit).*

Experiment:
Dilution linearity was assessed by serially diluting samples containing recombinant soluble Neuropilin-1 with STD1.

Data showing the dilution of recombinant Neuropilin-1 in **serum** samples

Sample ID	Neuropilin-1 [nmol/l]				R [%]		
	ref	1+1	1+3	1+7	1+1	1+3	1+7
#S1	6.1	3.3	1.6	0.8	108	102	110
#S2	6.8	3.4	1.6	0.8	99	92	90
#S3	7.8	3.1	2.0	0.9	79	100	97
			Mean R [%]		**95**	**98**	**99**

Data showing the dilution of recombinant Neuropilin-1 in **EDTA plasma** samples

Sample ID	Neuropilin-1 [nmol/l]				R [%]		
	ref	1+1	1+3	1+7	1+1	1+3	1+7
#E1	6.6	2.9	1.7	0.8	87	101	101
#E2	7.1	3.1	1.7	0.8	86	96	89
#E3	7.6	3.0	1.7	0.9	79	92	91
			Mean R [%]		**84**	**96**	**94**

Data showing the dilution of recombinant Neuropilin-1 in **citrate plasma** samples

Sample ID	Neuropilin-1 [nmol/l]				R [%]		
	ref	1+1	1+3	1+7	1+1	1+3	1+7
#C1	7.4	2.9	1.7	0.8	77	90	88

Data showing the dilution of recombinant Neuropilin-1 in **heparin plasma** samples

Sample ID	Neuropilin-1 [nmol/l]				R [%]		
	ref	1+1	1+3	1+7	1+1	1+3	1+7
#H1	6.9	3.3	1.8	0.9	95	104	104

PRECISION

Intra-assay precision & Inter-assay precision

Intra-assay (n=6) ≤ 11%, Inter-assay (n=) ≤ 10%

Intra-assay: 2 samples of known concentrations were tested 6 times with 1 kit lot by 1 operator.
Inter-assay: 2 samples of known concentrations were tested 12 times with 2 different kit lots on 3 days by 3 different operators.

Intra-assay (n=6)	Sample 1	Sample 2
Mean (nmol/l)	0.8	6.2
SD (nmol/l)	0.1	0.4
CV (%)	11	7

Inter-assay (n=12)	Sample 1	Sample 2
Mean (pmol/l)	0.8	6.2
SD (pmol/l)	0.08	0.36
CV (%)	10	6

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 total soluble Neuropilin-1 ELISA is **0.09 nmol/l**.

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]).
The LLOQ of the total soluble Neuropilin-1 ELISA is **0.09 nmol/l**.

SAMPLE STABILITY

Sample preparation

Collect venous blood samples by using standardized blood collection tubes. Perform serum and plasma separation by centrifugation according to supplier's instructions of the blood collection devices as soon as possible. The acquired serum and plasma samples should be measured as soon as possible. For longer storage aliquot samples and store at -25°C or lower for long time storage at -80°C. All samples should undergo only 5 freeze-thaw cycles.

Freeze/thaw of plasma samples containing endogenous soluble Neuropilin-1

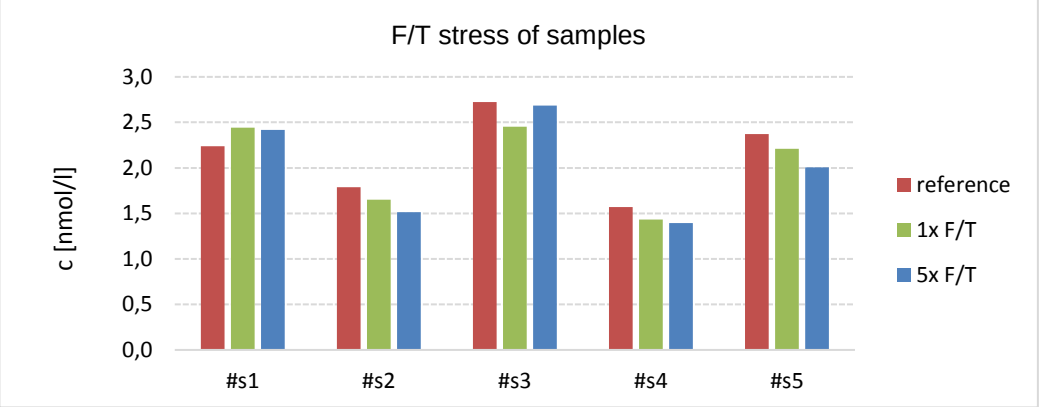
Five serum samples were aliquoted and freeze-thaw stressed. The reference samples are freeze-thawed once. Samples can undergo 5 freeze-thaw cycles. The mean recovery of sample concentrations stressed by 5 F/T cycles is 95%.

Samples can undergo 5 freeze-thaw cycles.

Data showing Neuropilin-1 concentrations of samples after freeze-thaw cycles:

Sample ID	Neuropilin-1 [nmol/l]			R [%] 5 F/T vs ref
	Reference	1x	5x	
#S1	2.2	2.4	2.4	108
#S2	1.8	1.7	1.5	85
#S3	2.7	2.5	2.7	99
#S4	1.6	1.4	1.4	89
#S5	2.4	2.2	2.0	85
			Mean R [%]	**93**

Graph showing F/T cycles of serum samples



CHARACTERIZATION OF THE ANTIBODIES

The total soluble Neuropilin-1 ELISA utilizes a monoclonal anti-human Neuropilin-1 antibody that binds to a linear epitope close to the N-terminus in the CUB 1 domain of the Neuropilin-1 molecule. The polyclonal detection antibody binds to multiple linear epitopes, distributed over the entire Neuropilin-1 molecule.

DAB Detection Antibody: Monoclonal mouse anti-Neuropilin-1 antibody-HRP binds to a linear epitope close to the N-terminus in the CUB 1 domain of the Neuropilin-1 molecule.

CAB Coating Antibody: Polyclonal sheep anti-human Neuropilin-1 antibody binding to multiple linear epitopes distributed over the entire Neuropilin-1 molecule (between AA81-AA630).

SPECIFICITY

The assay is optimized to detect soluble total Neuropilin-1 (NRP1) in human plasma and serum. The specific interaction of the analyte with the coating and the detection antibody was analysed. This assay recognizes endogenous (natural) and recombinant human soluble Neuropilin-1 (isoform 2 and 3).
The human sequence homology between NRP1 and NRP2 is low. The amino acid sequence in the respective binding regions of the antibodies show no homology with NRP2. Thus a cross reactivity to NRP2 is not expected.

ISOFORMS
Isoform1 is not soluble in blood.
Isoforms 2 (longer) and 3 (shorter, C-terminal domain missing), which are generated by alternative splicing, are both soluble and detected by this assay (cat. no. BI-20409).

CALIBRATION

This immunoassay is calibrated against recombinant human soluble Neuropilin-1 protein (AA22-AA644 of O14786-2 (Uniprot ID)).

VALIDATION GUIDELINES

The assay is fully validated for human serum and plasma samples according to ICH Q2 (R1) (8).

LITERATURE

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Available on our Website **www.bmgrp.com**

Instructions for use (IFU)
Protocol for urine samples
Protocol for cell culture supernatants samples
Protocol for non-human samples
Material Safety Data Sheet (MSDS)
Biomedica Neuropilin-1 ELISA – Info Leaflet

Date: May 2018

MEASUREMENT OF NEUROPILIN-1 in human URINE, CELL CULTURE SUPERNATANTS and NON-HUMAN SAMPLES

The following experiments have been performed to test the use of the Neuropilin-1 assay (cat. no. BI-20409) in human urine samples, human cell culture supernatants and non-human samples.

1. MEASUREMENT of NEUROPILIN-1 in HUMAN URINE SAMPLES

Note: the experiments performed to measure total soluble Neuropilin-1 in urine samples are not a full validation but are merely a performance check.

Performance check:

Urine samples (n=6) from apparently healthy subjects and from patients with kidney disease were assayed with the total soluble Neuropilin-1 ELISA following the standard protocol (including GuHCl treatment) using undiluted urine. Sample concentrations were calculated by using STD1-STD7.

- Endogenous Neuropilin-1 was undetectable in tested samples (cohorts from apparently healthy and kidney disease) thus no test on specificity (competition) was carried out.

- Urine samples can be spiked with recombinant human Neuropilin-1. The average recovery of 6 human urine samples is 105%.

- Urine samples spiked with recombinant human Neuropilin-1 can be diluted 1+1 with STD1 (0 nmol/l) that is supplied in the kit. The average recovery of 6 human urine samples is 94%.

RECOVERY

Recovery was assessed by adding STD7 (supplied in the kit, final concentration 6 nmol/l human recombinant Neuropilin-1) directly to 6 different human urine samples (ratio 1+1).

Data showing spike/recovery of human urine samples:

	Neuropilin-1 [nmol/l]		
Sample ID	**Reference**	**+ 6 nmol/l**	**S/R [%]**
#U1	0.1	6.1	100
#U2	0	6.3	105
#U3	0	6.5	108
#U4	0	6.3	105
#U5	0	6.5	108
#U6	0	6.3	105
		Mean R [%]	**105**

LINEARITY

Dilution linearity was assessed by diluting urine samples spiked with 6 nmol/l recombinant Neuropilin-1 with STD1 (0 nmol/l, supplied in the kit).

Data showing the dilution of recombinant Neuropilin-1 in urine samples:

Sample ID	Neuropilin-1 [nmol/l]		Dil R [%]
	Reference	Dil 1+1	
#U1	6.1	2.6	87
#U2	6.3	2.8	89
#U3	6.5	3.2	98
#U4	6.3	3.0	96
#U5	6.5	3.2	100
#U6	6.3	3.0	94
		Mean R [%]	**94**

Suggested protocol for the measurement of human Neuropilin-1 in urine samples

Follow standard protocol as indicated in the package insert:

In pre-dilution plate:
*Use **10 µl urine sample for GuHCl treatment**.*

Continue with protocol as indicated in the instructions for use.

In pre-coated plate:
*Transfer **50 µl pre-treated urine sample** from pre-dilution plate into respective wells. Swirl gently.*
For the transfer into the coated plate it is recommended to use a multichannel pipette. Transfer should be performed as soon as possible.

Continue with protocol as indicated in the instructions for use.

If required, dilute samples 1+1 with STD1 (provided in the kit) prior to GuHCl treatment.

2. MEASUREMENT of NEUROPILIN-1 in CELL CULTURE SUPERNATANTS

Note: the experiments performed to measure total soluble Neuropilin-1 in cell culture supernatants are not a full validation but are merely a performance check.

Performance Check:

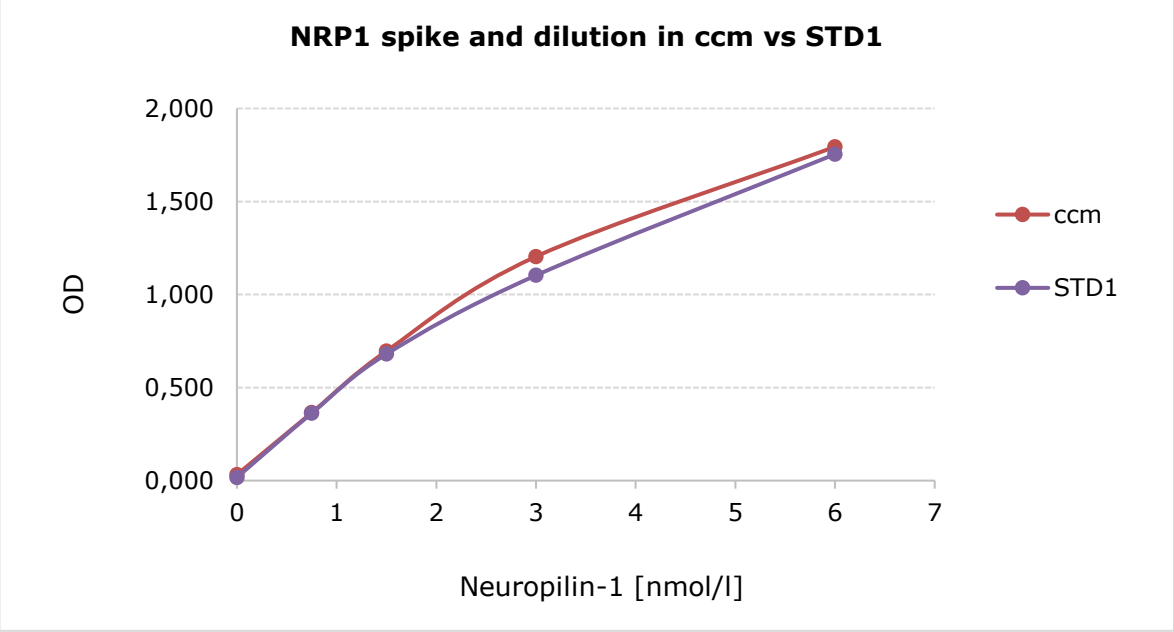
Cell culture medium (ccm: RPMI1640 containing 10% fetal calf serum) was tested undiluted and spiked by adding STD7 (provided in the kit, final concentration 6 nmol/l human recombinant Neuropilin-1) directly to cell culture medium (ratio 1+1). The spiked solution was diluted 1+1, 1+3 and 1+7 with the cell culture medium.

As a comparison, the spike recovery and dilution linearity of the standard matrix (=STD1, containing 0 nmol/l human recombinant Neuropilin-1) and the dilutions with cell culture medium is shown.

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

		Neuropilin-1 [nmol/l]				
Dilution medium	**Sample ID**	**Reference**	**+ 6 nmol/l**	**1+1**	**1+3**	**1+7**
ccm	ccm	0	6.0	3.2	1.6	0.8
ccm	STD1	0	5.8	2.8	1.5	0.7

Graph showing dilution of cell culture medium (ccm) and a comparison to the standard (STD1, containing 0 nmol/l human NRP1). Both ccm and STD1 were spiked with the same amount of human Neuropilin-1 (6 nmol/l).



Protocol for the measurement of human Neuropilin-1 in cell culture supernatants

Preparation of a cell culture medium (ccm) based standard curve:

Reconstitute STD7 in 200 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 medium 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 nmol/l).

NOTE: after preparation of the cell culture medium (ccm) based standard curve, proceed with the protocol as indicated in the instructions for use. ALL samples including the ccm based standards must be treated with GuHCl before assaying.

Attention: Concentrations defined for CTRL A and B are only valid for measuring total soluble Neuropilin-1 in human serum or plasma. The controls cannot be used for cell culture measurements.

Suggested protocol for the measurement of human Neuropilin-1 in cell culture supernatants (cc)

Follow standard protocol as indicated in the package insert:

In pre-dilution plate:
*Use **10 µl cc sample for GuHCl treatment**.*

In pre-coated plate:
*Transfer **50 µl pre-treated cc sample** from pre-dilution plate into respective wells.*
Swirl gently.
For the transfer into the coated plate it is recommended to use a multichannel pipette.
Transfer should be performed as soon as possible.

If required, dilute samples 1+1 with ccm (cell culture medium) prior to GuHCl treatment.

3. MEASUREMENT of total soluble NEUROPILIN-1 in NON-HUMAN SAMPLES

Note: the experiments performed to measure total soluble Neuropilin-1 in non-human samples are not a full validation but are merely a performance check.

The sequence of Neuropilin-1 in mammals is highly conserved.

The listed species below show a homology of >90% with human Neuropilin-1:

- Macaca mulatta (Rhesus macaque)
- Pan paniscus (Pygmy chimpanzee) (Bonobo)
- Pongo abelii (Sumatran orangutan) (Pongo pygmaeus abelii)
- Sus scrofa (Pig)
- Chlorocebus sabaeus (Green monkey) (Cercopithecus sabaeus)
- Aotus nancymaae (Ma's night monkey)
- Gorilla gorilla gorilla (Western lowland gorilla)
- Neovison vison (American mink) (Mustela vison)
- Callithrix jacchus (White-tufted-ear marmoset)
- And more

Table: Sequence comparison of NRP-1 protein in mouse, human, and rat

```
P97333 NRP1_MOUSE            1   MERGLPLLCATLALALALAGAFRSDKCGGTIKIENPGYLTSPGYPHSYHPSEKCEWLIQA       60
O14786 NRP1_HUMAN            1   MERGLPLLCAVLALVLAPAGAFRNDKCGDTIKIESPGYLTSPGYPHSYHPSEKCEWLIQA       60
Q9QWJ9 NRP1_RAT              1   MERGLPLLCATLALALALAGAFRSDKCGGTIKIENPGYLTSPGYPHSYHPSEKCEWLIQA       60
                                 **********.***.** *****.****.*****.*************************

P97333 NRP1_MOUSE           61   PEPYQRIMINFNPHFDLEDRDCKYDYVEVIDGENEGGRLWGKFCGKIAPSPVVSSGPFLF       120
O14786 NRP1_HUMAN           61   PDPYQRIMINFNPHFDLEDRDCKYDYVEVFDGENENGHFRGKFCGKIAPPPVVSSGPFLF       120
Q9QWJ9 NRP1_RAT             61   PEPYQRIMINFNPHFDLEDRDCKYDYVEVIDGENEGGRLWGKFCGKIAPSPVVSSGPFLF       120
                                 *:*************************:*****.*:: ********* **********

P97333 NRP1_MOUSE          121   IKFVSDYETHGAGFSIRYEIFKRGPECSQNYTAPTGVIKSPGFPEKYPNSLECTYIIFAP       180
O14786 NRP1_HUMAN          121   IKFVSDYETHGAGFSIRYEIFKRGPECSQNYTTPSGVIKSPGFPEKYPNSLECTYIVFVP       180
Q9QWJ9 NRP1_RAT            121   IKFVSDYETHGAGFSIRYEIFKRGPECSQNYTAPTGVIKSPGFPEKYPNSLECTYIIFAP       180
                                 **********************************:*:**********************:*.*

P97333 NRP1_MOUSE          181   KMSEIILEFESFDLEQDSNPPGGMFCRYDRLEIWDGFPEVGPHIGRYCGQKTPGRIRSSS       240
O14786 NRP1_HUMAN          181   KMSEIILEFESFDLEPDSNPPGGMFCRYDRLEIWDGFPDVGPHIGRYCGQKTPGRIRSSS       240
Q9QWJ9 NRP1_RAT            181   KMSEIILEFESFDLEQDSNPPGGVFCRYDRLEIWDGFPEVGPHIGRYCGQKTPGRIRSSS       240
                                 *************** ********:**************:**********************

P97333 NRP1_MOUSE          241   GVLSMVFYTDSAIAKEGFSANYSVLQSSISEDFKCMEALGMESGEIHSDQITASSQYGTN       300
O14786 NRP1_HUMAN          241   GILSMVFYTDSAIAKEGFSANYSVLQSSVSEDFKCMEALGMESGEIHSDQITASSQYSTN       300
Q9QWJ9 NRP1_RAT            241   GILSMVFYTDSAIAKEGFSANYSVLQSSISEDFKCMEALGMESGEIHSDQITASSQYGTN       300
                                 *:*************************:**************************.**

P97333 NRP1_MOUSE          301   WSVERSRLNYPENGWTPGEDSYKEWIQVDLGLLRFVTAVGTQGAISKETKKKYYVKTYRV       360
O14786 NRP1_HUMAN          301   WSAERSRLNYPENGWTPGEDSYREWIQVDLGLLRFVTAVGTQGAISKETKKKYYVKTYKI       360
Q9QWJ9 NRP1_RAT            301   WSVERSRLNYPENGWTPGEDSYREWIQVDLGLLRFVTAVGTQGAISKETKKKYYVKTYRV       360
                                 **.*******************:**********************************::

P97333 NRP1_MOUSE          361   DISSNGEDWISLKEGNKAIIFQGNTNPTDVVLGVFSKPLITRFVRIKPVSWETGISMRFE       420
O14786 NRP1_HUMAN          361   DVSSNGEDWITIKEGNKPVLFQGNTNPTDVVVAVFPKPLITRFVRIKPATWETGISMRFE       420
Q9QWJ9 NRP1_RAT            361   DISSNGEDWITLKEGNKAIIFQGNTNPTDVVFGVFPKPLITRFVRIKPASWETGISMRFE       420
                                 *:********::***** ::***********..** ************.:**********

P97333 NRP1_MOUSE          421   VYGCKITDYPCSGMLGMVSGLISDSQITASNQADRNWMPENIRLVTSRTGWALPPSPHPY       480
O14786 NRP1_HUMAN          421   VYGCKITDYPCSGMLGMVSGLISDSQITSSNQGDRNWMPENIRLVTSRSGWALPPAPHSY       480
Q9QWJ9 NRP1_RAT            421   VYGCKITDYPCSGMLGMVSGLISDSQITASNQGDRNWMPENIRLVTSRTGWALPPSPHPY       480
                                 ****************************:***.***************:******:** *

P97333 NRP1_MOUSE          481   TNEWLQVDLGDEKIVRGVIIQGGKHRENKVFMRKFKIAYSNNGSDWKTIMDDSKRKAKSF       540
O14786 NRP1_HUMAN          481   INEWLQIDLGEEKIVRGIIIQGGKHRENKVFMRKFKIGYSNNGSDWKMIMDDSKRKAKSF       540
Q9QWJ9 NRP1_RAT            481   INEWLQVDLGDEKIVRGVIIQGGKHRENKVFMRKFKIAYSNNGSDWKMIMDDSKRKAKSF       540
                                  *****:***:******:******************.********* ************

P97333 NRP1_MOUSE          541   EGNNNYDTPELRTFSPLSTRFIRIYPERATHSGLGLRMELLGCEVEAPTAGPTTPNGNPV       600
O14786 NRP1_HUMAN          541   EGNNNYDTPELRTFPALSTRFIRIYPERATHGGLGLRMELLGCEVEAPTAGPTTPNGNLV       600
Q9QWJ9 NRP1_RAT            541   EGNNNYDTPELRAFTPLSTRFIRIYPERATHSGLGLRMELLGCEVEVPTAGPTTPNGNPV       600
                                 ************:*  **************.**************.********** *

P97333 NRP1_MOUSE          601   DECDDDQANCHSGTGDDFQLTGGTTVLATEKPTIIDSTIQSEFPTYGFNCEFGWGSHKTF       660
O14786 NRP1_HUMAN          601   DECDDDQANCHSGTGDDFQLTGGTTVLATEKPTVIDSTIQSEFPTYGFNCEFGWGSHKTF       660
Q9QWJ9 NRP1_RAT            601   DECDDDQANCHSGTGDDFQLTGGTTVLATEKPTIIDSTIQSEFPTYGFNCEFGWGSHKTF       660
                                 **********************************:*************************

P97333 NRP1_MOUSE          661   CHWEHDSHAQLRWSVLTSKTGPIQDHTGDGNFIYSQADENQKGKVARLVSPVVYSQSSAH       720
O14786 NRP1_HUMAN          661   CHWEHDNHVQLKWSVLTSKTGPIQDHTGDGNFIYSQADENQKGKVARLVSPVVYSQNSAH       720
Q9QWJ9 NRP1_RAT            661   CHWEHDSHAQLRWRVLTSKTGPIQDHTGDGNFIYSQADENQKGKVARLVSPVVYSQSSAH       720
                                 ******.*.**:* ***************************************.***

P97333 NRP1_MOUSE          721   CMTFWYHMSGSHVGTLRVKLRYQKPEEYDQLVWMVVGHQGDHWKEGRVLLHKSLKLYQVI       780
O14786 NRP1_HUMAN          721   CMTFWYHMSGSHVGTLRVKLRYQKPEEYDQLVWMAIGHQGDHWKEGRVLLHKSLKLYQVI       780
Q9QWJ9 NRP1_RAT            721   CMTFWYHMSGSHVGTLRVKLHYQKPEEYDQLVWMVVGHQGDHWKEGRVLLHKSLKLYQVI       780
                                 ********************:*************.:************************

P97333 NRP1_MOUSE          781   FEGEIGKGNLGGIAVDDISINNHISQEDCAKPTDLDKKNTEIKIDETGSTPGYEGEGEGD       840
O14786 NRP1_HUMAN          781   FEGEIGKGNLGGIAVDDISINNHISQEDCAKPADLDKKNPEIKIDETGSTPGYEGEGEGD       840
Q9QWJ9 NRP1_RAT            781   FEGEIGKGNLGGIAVDDISINNHIPQEDCAKPTDLDKKNTEIKIDETGSTPGYE-EGKGD       839
                                 ********************** ********:****** ************** **:**

P97333 NRP1_MOUSE          841   KNISRKPGNVLKTLDPILITIIAMSALGVLLGAVCGVVLYCACWHNGMSERNLSALENYN       900
O14786 NRP1_HUMAN          841   KNISRKPGNVLKTLDPILITIIAMSALGVLLGAVCGVVLYCACWHNGMSERNLSALENYN       900
Q9QWJ9 NRP1_RAT            840   KNISRKPGNVLKTLDPILITIIAMSALGVLLGAVCGVVLYCACWHNGMSERNLSALENYN       899
                                 ************************************************************

P97333 NRP1_MOUSE          901   FELVDGVKLKKDKLNPQSNYSEA                                          923
O14786 NRP1_HUMAN          901   FELVDGVKLKKDKLNTQSTYSEA                                          923
Q9QWJ9 NRP1_RAT            900   FELVDGVKLKKDKLNPQSNYSEA                                          922
                                 *************** **.****
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Performance check:

3 mouse serum samples, 3 rat serum samples and 2 pig serum samples (all sera derived from apparently healthy animals) were tested undiluted and spiked by adding STD7 (final concentration 6 nmol/l of human recombinant Neuropilin-1) directly to all samples (ratio 1+1). The spiked sera were diluted 1+1 and 1+3 with STD1 (containing 0 nmol/l NRP1, supplied in the kit). Sample concentrations were calculated by using STD1-STD7.

- Endogenous Neuropilin-1 was undetectable in tested samples thus no test on specificity (competition) was carried out.

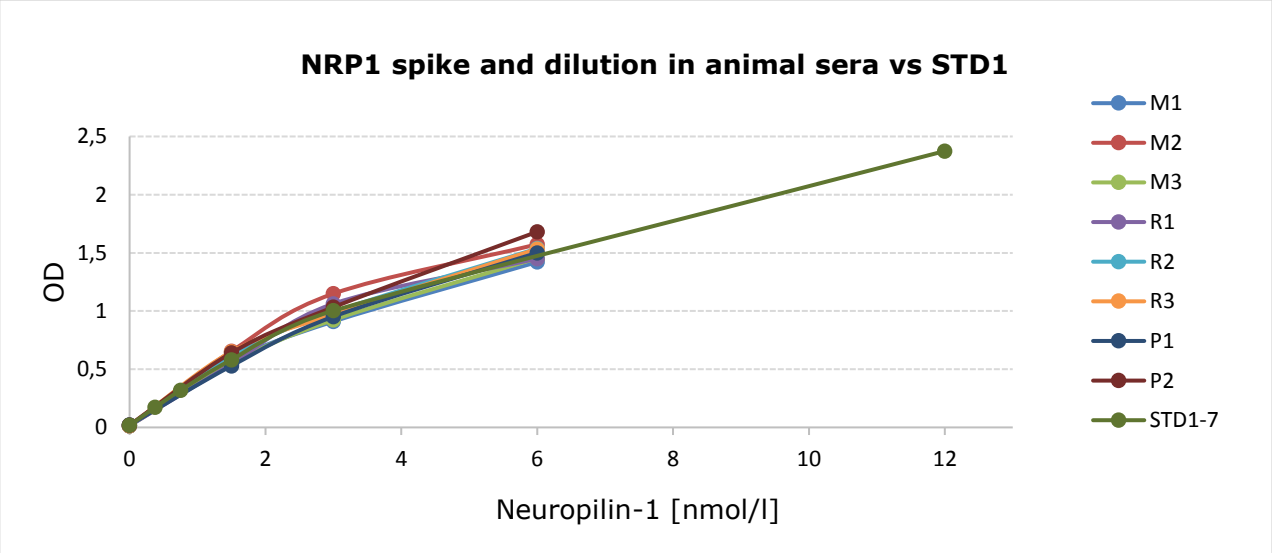
- Animal sera can be spiked with recombinant human NRP1 (final concentration 6 nmol/l) – the average recovery is 90%.

- Spiked animal samples can be diluted 1+1 and 1+3 with STD1 (0 nmol/l) – the average recovery is 111% and 119%.

Table: calculation of animal sample concentrations, and spike recovery

Sample matrix	ID	Ref	NRP1 +6 nmol/l	S/R [%]	dil 1+1	dil 1+3	R [%] 1+1	R [%] 1+3
mouse serum	M1	0	4.9	81	2.6	1.5	108	121
mouse serum	M2	0	5.7	95	3.6	1.7	126	123
mouse serum	M3	0	5.0	84	2.7	1.4	107	112
rat serum	R1	0	5.0	84	3.2	1.4	129	112
rat serum	R2	0	5.5	92	2.9	1.6	107	119
rat serum	R3	0	5.5	91	2.9	1.7	105	127
pig serum	P1	0	5.3	88	2.8	1.4	106	102
pig serum	P2	0	6.3	106	3.1	1.7	98	107
			Mean R [%]	**90**			**111**	**119**

Graph: Neuropilin-1 standard curve (STD1-7) and dilution curves of animal sera spiked with human NRP1 diluted with STD1 (0 nmol/l NRP1)



Suggested protocol for the measurement of human Neuropilin-1 in non-human samples

Follow standard protocol as indicated in the package insert:

In pre-dilution plate:
*Use **10 µl non-human sample for GuHCl treatment**.*

Continue with protocol as indicated in the instructions for use.

In pre-coated plate:
*Transfer **50 µl pre-treated non-human sample** from pre-dilution plate into respective wells. Swirl gently.*
For the transfer into the coated plate it is recommended to use a multichannel pipette. Transfer should be performed as soon as possible.

Continue with protocol as indicated in the instructions for use.

If required, dilute samples 1+1 with STD1 (provided in the kit) prior to GuHCl treatment.