

Vanin-1 Mouse/Rat ELISA

for the quantitative determination of mouse or rat Vanin-1 in serum, plasma or urine
Cat. No. BI-VAN1MR. 12 x 8 tests

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

CONTENTS

ASSAY CHARACTERISTICS Summary.....	2
PRODUCT OVERVIEW.....	3
TYPICAL STANDARD CURVE.....	3
PRINCIPLE OF THE ASSAY.....	3
SAMPLE VALUES.....	4
Vanin-1 Values.....	4
ASSAY PERFORMANCE CHARACTERISTICS.....	4
ACCURACY.....	4
DILUTION LINEARITY & PARALLELISM.....	5
Parallelism.....	7
Dilution Linearity.....	8
PRECISION.....	9
Within-Run Precision (Intra-Assay).....	9
In-Between-Run Precision (Inter-Assay).....	9
DETECTION LIMIT & SENSITIVITY.....	9
SAMPLE STABILITY.....	10
Sample Collection and Storage.....	10
Freeze-Thaw Stability of Samples Containing Endogenous Vanin-1.....	10
STANDARD STABILITY.....	10
SPECIFICITY.....	10
Competition of Signal.....	10
CALIBRATION.....	11
Additional Documents Available Online (www.bmgrp.com).....	11

ASSAY CHARACTERISTICS Summary

Method	Sandwich ELISA, HRP/TMB, 12x8-well detachable strips		
Sample type(s)	Serum, plasma and urine		
Sample volume	5 µl / well		
Assay time	4 hours/ 30 mins		
Standard range	0 – 200 pmol/l 0 – 10.6 ng/ml		
Conversion factor	1 ng/ml = 19.2 pmol/l (MW: 52.07 kDa)		
Sensitivity	LOD: 2.31 pmol/l; LLOQ: 6.25 pmol/l		
Precision		n	Average % CV
	Within-run	6	≤8
	In-between-run	5	≤8
Accuracy (Spike/Recovery of rec. Vanin-1)		n	Average % recovery
	Mouse	7	93
	Rat	4	94
Dilution linearity of endogenous Vanin-1		n	Average % of expected dilution
			1+1 1+3
	Mouse	4	97 84
	Rat	3	92 -
Specificity	Endogenous and recombinant Vanin-1 .		
Use	Research use only		
Vanin-1 reference values		n	Median Vanin-1 c[pmol/l]
	Mouse serum	5	22
	Mouse plasma	5	24
	Mouse urine	6	21
	Rat serum	7	7
	Rat plasma	8	7

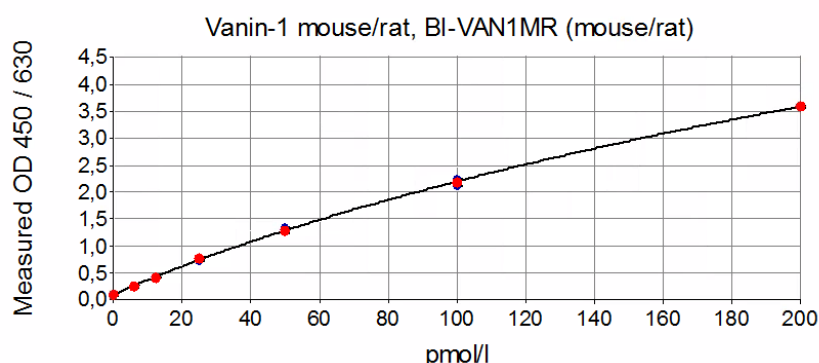
PRODUCT OVERVIEW

The Vanin-1 mouse/rat immunoassay is a 4.5 hour, 96-well sandwich ELISA for the quantitative determination of mouse or rat Vanin-1 in serum, plasma and urine. The kit utilizes recombinant mouse Vanin-1 as a calibrator. The VNN1 gene is conserved in chimpanzee, rhesus monkey, dog, cow, mouse, rat, and chicken

<https://www.ncbi.nlm.nih.gov/homologene/32130> .

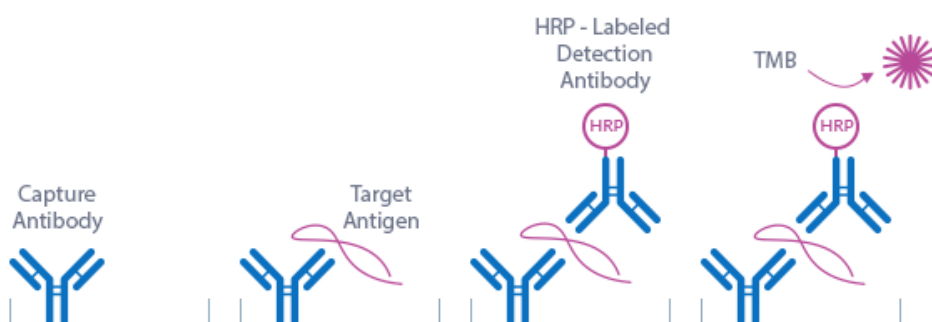
TYPICAL STANDARD CURVE

The figure below shows a typical standard curve for the Vanin-1 Mouse/Rat ELISA. The immunoassay is calibrated against recombinant mouse Vanin-1 peptide:



PRINCIPLE OF THE ASSAY

The Vanin-1 mouse/rat ELISA is a sandwich enzyme immunoassay for the quantitative determination of Vanin-1 in mouse and rat samples.



In a first step, assay buffer is pipetted into the wells of the microtiter strips. Thereafter, standard/control/sample and detection antibody (polyclonal sheep anti-mouse Vanin-1-HRPO) are pipetted into the wells, which are pre-coated with anti-mouse Vanin-1 antibody. Vanin-1 present in the standard/control/sample binds to the pre-coated antibody in the well and forms a sandwich with the detection antibody. In the washing step, all non-specific unbound material is removed. In a next step, the substrate (TMB, tetramethylbenzidine) is pipetted into the wells. The enzyme-catalyzed color change of the substrate is directly proportional to the amount of Vanin-1 present in the sample. This color change is detectable with a standard microplate reader. A dose response curve of the absorbance (optical density, OD at 450 nm)

versus standard concentration is generated using the values obtained from the standards. The concentration of Vanin-1 in the sample is determined directly from the dose response curve.

SAMPLE VALUES

Vanin-1 Values

To provide reference values for circulating mouse and rat Vanin-1, a panel of samples was tested. A summary of the results is shown below:

Sample matrix	n	Vanin-1 [pmol/l]			
		Mean	Median	Minimum	Maximum
Mouse serum	5	24	22	9	39
Mouse plasma	5	25	24	19	34
Mouse urine	6	26	21	3	62
Rat serum	8	7	7	6	11
Rat plasma	8	8	7	5	16

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

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 Vanin-1 Mouse/Rat ELISA was measured by adding recombinant Mouse Vanin-1 to mouse or rat samples containing a known concentration of endogenous Vanin-1. The %recovery of the spiked concentration was calculated as the percentage of measured compared over the expected value.

This table shows the summary of the recovery experiments in the Vanin-1 mouse/rat ELISA in different sample matrices:

Samples	n	Spike/Recovery [%]			
		+25 pmol/l		+100 pmol/l	
		Mean	Range	Mean	Range
Mouse	7	93	87-124	90	79-102
Rat	4	94	68-103	87	78-96

Experiments:

Recovery of spiked samples was tested by adding two concentrations of mouse recombinant Vanin-1 (25 pmol/l and 100 pmol/l) to mouse and rat samples.

Data showing recovery of recombinant mouse Vanin-1 in mouse serum samples:

Sample ID	Spike Vanin-1 [pmol/l]			S/R [%]	
	0	25	100	25	100
MS1	10	27	84	87	79
MS2	25	36	106	94	93
MS3	43	44	107	91	85
MS4	27	37	103	94	89
Mean S/R [%]				92	87
Min				87	79
Max				94	93

Data showing recovery of recombinant mouse Vanin-1 in a mouse urine samples:

Sample ID	Spike Vanin-1 [pmol/l]			S/R [%]	
	0	25	100	25	100
MU1	32	47	107	124	91
MU2	22	34	101	93	90
MU3	37	41	121	90	102
Mean S/R [%]				93	91
Min				90	90
Max				124	102

Data showing recovery of recombinant mouse Vanin-1 in rat plasma samples:

Sample ID	Spike Vanin-1 [pmol/l]			S/R [%]	
	0	25	100	25	100
RP1	6	26	81	92	78
RP2	3	19	85	68	83
RP3	8	28	100	95	96
RP4	10	31	96	103	91
Mean S/R [%]				94	87
Min				68	78
Max				103	95

DILUTION LINEARITY & PARALLELISM

Tests of dilution linearity and parallelism ensure that both endogenous and recombinant samples containing Vanin-1 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

Experiment:

Dilution linearity was assessed by serially diluting samples spiked with 100 pmol/l recombinant mouse Vanin-1 with assay buffer.

Summary table below shows the mean recovery and range of serially diluted recombinant mouse Vanin-1 in several sample matrices:

Sample matrix	n	Recovery [%]					
		1+1		1+3		1+7	
		Mean	Range	Mean	Range	Mean	Range
Mouse	4	85	80-97	80	73-83	75	62-96
Rat	3	93	88-105	110	106-119	126	115-136

Detailed data of recovery of recombinant Mouse Vanin-1 in diluted samples are shown below:

Data showing dilution linearity of 100 pmol/l recombinant mouse Vanin-1 spiked into mouse serum samples (ref) containing endogenous Vanin-1:

Sample ID	Mouse Vanin-1 [pmol/l]				Recovery [%]		
	Ref	1+1	1+3	1+7	1+1	1+3	1+7
MS1	104	42	19	9	80	73	71
MS2	121	49	24	15	81	80	96
MS3	102	46	20	8	90	80	62
MS4	112	55	23	11	97	83	80
Mean R [%]					85	80	75
Min					80	73	62
Max					97	83	96

Data showing dilution linearity of 100 pmol/l recombinant mouse Vanin-1 spiked into rat plasma (ref) containing endogenous Vanin-1:

Sample ID	Mouse Vanin-1 [pmol/l]				Recovery [%]		
	Ref	1+1	1+3	1+7	1+1	1+3	1+7
RP1	82	43	22	12	105	106	115
RP2	92	43	25	15	93	110	126
RP3	89	39	27	15	88	119	136
Mean R [%]					93	110	126
Min					88	106	115
Max					105	119	136

Parallelism

Experiment:

Parallelism was assessed by serially diluting samples containing **endogenous** Vanin-1 with assaybuffer.

Summary table below show the mean recovery and range of serially diluted endogenous Vanin-1 in several sample matrices:

Sample matrix	n	Recovery [%]					
		1+1		1+3		1+7	
		Mean	Range	Mean	Range	Mean	Range
Mouse	4	97	84-103	84	71-94	95	85-105
Rat	3	92	87-106	-	-	-	-

Detailed data of recovery of endogenous Mouse Vanin-1 in diluted samples are shown below:

Data showing parallelism of endogenous Vanin-1 in mouse serum samples:

Sample ID	Vanin-1 [pmol/l]				Recovery [%]		
	Ref	1+1	1+3	1+7	1+1	1+3	1+7
MS1	43	20	9	5	92	81	92
MS2	27	11	5	3	84	71	85
MS3	32	17	7	4	103	87	105
MS4	37	19	9	5	102	94	98
Mean R [%]					97	84	95
Min					84	71	85
Max					103	94	105

Data showing parallelism of endogenous Vanin-1 in human urine samples:

Sample ID	Vanin-1 [pmol/l]				Recovery [%]		
	Ref	1+1	1+3	1+7	1+1	1+3	1+7
RP1	14	7	-	-	92	-	-
RP2	7	3	-	-	87	-	-
RP3	10	5	-	-	106	-	-
Mean R [%]					92	-	-
Min					87	-	-
Max					106	-	-

Dilution Linearity

Experiment:

Dilution linearity was assessed by serially diluting samples spiked with 100 pmol/l **recombinant** mouse Vanin-1 with assay buffer.

Summary table below shows the mean recovery and range of serially diluted recombinant mouse Vanin-1 in several sample matrices:

Sample matrix	n	Recovery [%]					
		1+1		1+3		1+7	
		Mean	Range	Mean	Range	Mean	Range
Mouse	4	117	103-124	111	101-118	104	84-129
Rat	3	108	95-114	84	74-99	88	87-93

Detailed data of recovery of recombinant mouse Vanin-1 in diluted samples are shown below:

Data showing dilution linearity of 100 pmol/l recombinant mouse Vanin-1 spiked into mouse serum samples (ref) containing endogenous Vanin-1:

Sample ID	Vanin-1 [pmol/l]				Recovery [%]		
	Ref	1+1	1+3	1+7	1+1	1+3	1+7
MS1	104	42	19	9	124	109	104
MS2	121	49	24	15	123	101	84
MS3	102	46	20	8	112	113	129
MS4	112	55	23	11	103	118	103
Mean R [%]					117	111	104
Min					103	101	84
Max					124	118	129

Data showing dilution linearity of 100 pmol/l recombinant mouse Vanin-1 spiked into rat plasma samples (ref) containing endogenous Vanin-1:

Sample ID	Vanin-1 [pmol/l]				Recovery [%]		
	Ref	1+1	1+3	1+7	1+1	1+3	1+7
RP1	82	43	22	12	95	99	93
RP2	92	43	25	15	108	84	87
RP3	89	39	27	15	114	74	88
Mean R [%]					108	84	88
Min					95	74	87
Max					114	99	93

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 (Intra-Assay)

Experiment:

Within-run / intra-assay precision was assessed by measuring 2 samples of known concentrations 6 times within one kit lot by one operator.

Within-run (n=6)	Sample 1	Sample 2
Mean (pmol/l)	8	20
SD (pmol/l)	0.6	1.6
CV (%)	8	8

In-Between-Run Precision (Inter-Assay)

Experiment:

In-between-run / inter-assay precision was assessed by measuring 2 samples 5 times within 2 kit lots by 2 different operators.

In-between run (n=5)	Sample 1	Sample 2
Mean (pmol/l)	63	41
SD (pmol/l)	4.0	3.1
CV (%)	6	8

DETECTION LIMIT & SENSITIVITY

To determine the sensitivity of the Vanin-1 mouse/rat 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 above the background signal, *i.e.* the signal that is measured in the absence of Vanin-1, with a confidence level of 99%. It is defined as the mean back calculated concentration of standard 1 (0 pmol/l of Vanin-1, 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 Vanin-1, 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 Vanin-1 mouse/rat ELISA:

LOD	2.31 pmol/l
LLOQ	6.25 pmol/l

SAMPLE STABILITY

Sample Collection and Storage

Serum, plasma and urine are suitable for use in this assay. Do not change sample type during studies. We recommend duplicate measurements for all samples, standards and controls. The sample collection and storage conditions listed are intended as general guidelines.

Blood samples:

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 and measure the acquired serum or plasma samples as soon as possible or aliquot and store at -25°C or lower.

Collect urine of the day, voided directly into a sterile container. Assay immediately or aliquot and store at -25°C or lower.

Freeze-Thaw Stability of Samples Containing Endogenous Vanin-1

The stability of endogenous Vanin-1 was tested by comparing measurements in blood samples that had undergone four freeze-thaw cycles (F/T). The mean recovery of sample concentration after 4 freeze-thaw cycles is 92%. Samples can undergo at least up to 4 freeze-thaw cycles.

STANDARD STABILITY

The stability of recombinant mouse Vanin-1 was tested by comparing 3 measurements in standards spiked to different values that had undergone 5 freeze-thaw cycles.

The mean recovery of standard concentration after 5 freeze-thaw cycles is 117%.

Sample matrix	Vanin-1 [pmol/l]				% Recovery after 5 freeze/thaw cycles
	Ref	1x	3x	5x	
Standard S1	34	42	40	47	138
Standard S2	75	84	87	88	117
Standard S3	166	180	176	183	110
				Mean R [%]	117

Standards can undergo at least up to 5 freeze-thaw cycles.

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 out by pre-incubating mouse and rat samples with an excess of coating antibody. The concentration measured in this mixture was then compared to a reference value, which was obtained from the same sample but without the pre-incubation step. Mean competition was 99%.

ID	Vanin-1 [pmol/l]		Recovery [%] Competition
	Reference	Reference + CAB	
M1	31	0	100
M2	49	0	100
M3	36	0	100
M4	14	0	100
R1	11	1	95
R2	14	0	97
R3	13	0	100
R4	11	0	100
Mean Comp. [%]			99

CALIBRATION

The Vanin-1 Mouse/Rat ELISA is calibrated against mouse Vanin-1 protein (<https://www.uniprot.org/uniprot/Q9Z0K8>).

Additional Documents Available Online (www.bmgrp.com)

Instructions for Use (IFU, package insert)
Material Safety Data Sheet (MSDS)