Human Vanin-1 (urine) ELISA

for the quantitative determination of human Vanin-1 in urine

Cat. No. BI-VAN1U. 12 x 8 tests

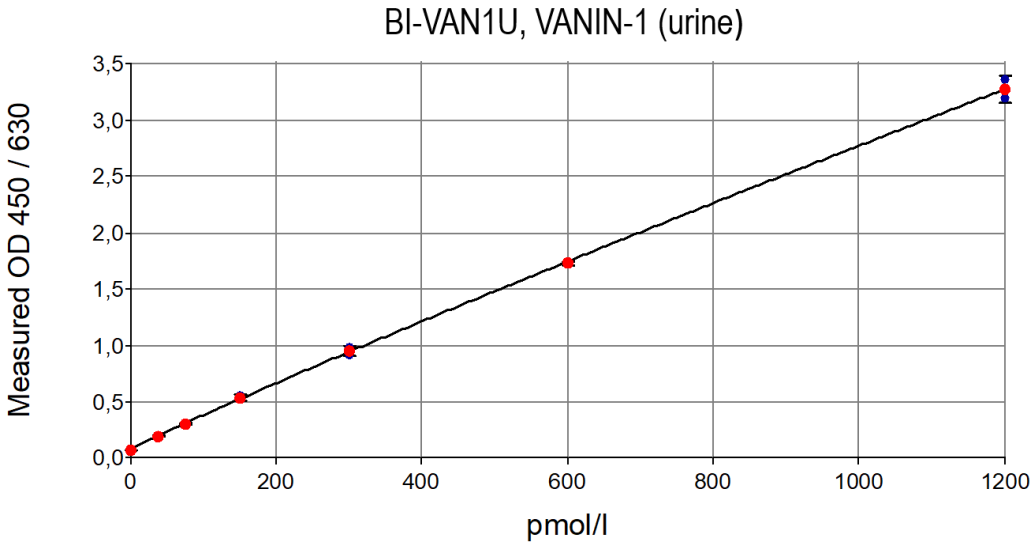
FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

CONTENTS

ASSAY CHARACTERISTICS Summary

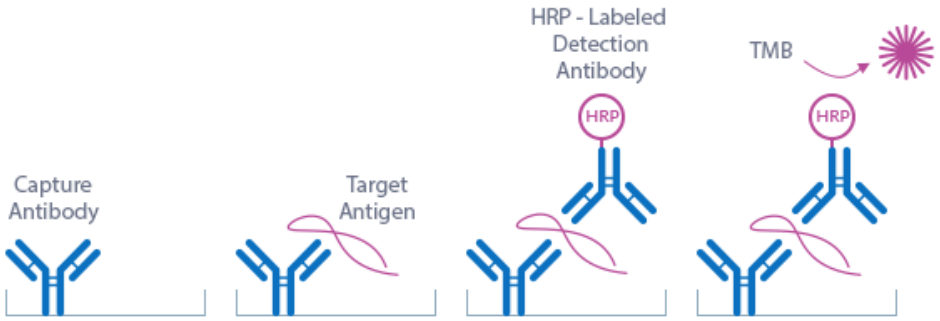
Method	Sandwich ELISA, HRP/TMB, 12x8-well detachable strips			
Sample type	Urine			
Sample volume	10 µl / well			
Assay time	4 h / 30 min			
Detection limit	9.6 pmol/l (500 pg/ml)			
Standard range	0 – 1,200 pmol/l (0 – 62,500 pg/ml)			
Conversion factor	1 pg/ml = 0.0192 pmol/l; MW: 52.07 kDa			
Precision		**n**	**CV [%]**	
	Within-run	3	≤5	
	In-between-run	9	≤7	
			Recovery [%]	
	Urine	6	93	
Dilution linearity of endogenous Vanin-1		**n**	**Recovery of expected dilution [%]**	
			1+1	**1+3**	**1+7**
	Urine	6	94	92	86
Specificity	Endogenous and recombinant human Vanin-1.			
Use	Research use only.			
Values of apparently healthy donors		**n**	**Median Vanin-1 [pmol/l]**	
	Urine	27	24.4	
		n	**Median Vanin-1 [pg/mg Creatinine]**	
	Urine	27	1131	

TYPICAL STANDARD CURVE



PRINCIPLE OF THE ASSAY

The Vanin-1 (urine) ELISA kit is a sandwich enzyme immunoassay for the quantitative determination of Vanin-1 in human urine.



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-human Vanin-1-HRP) are pipetted into the wells, which are pre-coated with polyclonal sheep anti-human 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 in Apparently Healthy Individuals

To provide values for Vanin-1 in apparently healthy individuals, a panel of urine samples was tested. In addition, these values were converted from pmol/l into pg/ml (conversion factor: 1 pg/ml= 0.0192 pmol/l) and normalized to Creatinine values.

	Vanin-1 [pmol/l]	Vanin-1 [pg/mg Creatinine]
	Urine	Urine
# of samples	n=27	n=27
Mean	196	1244
Median	**116**	**1131**
Minimum	3	77
Maximum	963	2813

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

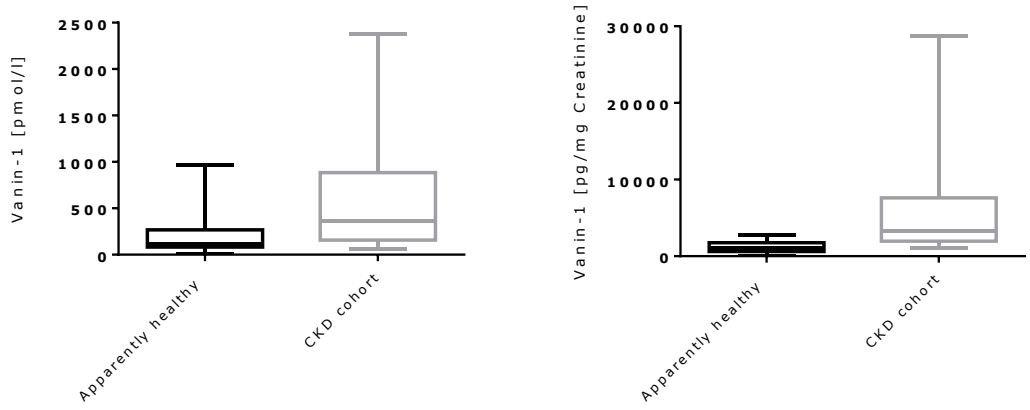
Vanin-1 Values in Individuals with Kidney Disease

Vanin-1 values were measured in a panel of urine samples from a kidney disease cohort (CKD). In addition, these values were converted from pmol/l into pg/ml (conversion factor: 1 pg/ml= 0.0192 pmol/l) and normalized to Creatinine values.

Summary of the results obtained with several disease panels:

	Vanin-1 [pmol/l]	Vanin-1 [pg/mg Creatinine]
	CKD, Urine	CKD, Urine
# of samples	n=24	n=24
Mean	589	5690
Median	**360**	**3280**
Minimum	57	1101
Maximum	2375	28764

Comparison of Vanin-1 Values in Apparently Healthy Individuals and Individuals with Kidney Disease:



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 human Vanin-1 (urine) ELISA was measured by adding recombinant Vanin-1 to human urine 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 human Vanin-1 (urine) ELISA in human urine samples:

Sample matrix	n	Spike/Recovery [%]			
		+120 pmol/l		+600 pmol/l	
		Mean	Range	Mean	Range
Urine	6	81	73-92	93	86-99

Experiments:
Recovery of spiked samples was tested by adding two concentrations of human recombinant Vanin-1 (120 pg/ml and 600 pmol/l) to human urine samples.

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

Sample ID	Spike Vanin-1 [pmol/l]			S/R [%]	
	0	120	600	160	600
U1	44	149	588	92	94
U2	62	156	597	84	94
U3	104	157	595	79	90
U4	19	99	527	73	86
U5	229	232	673	79	93
U6	86	147	637	79	99
			Mean S/R [%]	**81**	**93**
			Min	73	86
			Max	92	99

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 human samples spiked with known concentrations of recombinant analyte. By contrast, parallelism refers to dilution linearity in human samples and provides evidence that the endogenous analyte behaves in the same way as the recombinant one. Dilution linearity and parallelism are assessed for each sample type and should be within 20% of the expected concentration.

Parallelism

Experiment:
Parallelism was assessed by serially diluting human urine samples containing **endogenous** Vanin-1 with assay buffer.

Summary table below shows the mean recovery and range of serially diluted endogenous Vanin-1 in human urine:

Sample matrix	n	Recovery [%]					
		1+1		1+3		1+7	
		Mean	Range	Mean	Range	Mean	Range
Urine	6	94	85-100	92	79-98	86	69-99

Data showing dilution linearity 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
U1	1188	593	291	146	100	98	99
U2	717	357	169	83	100	94	93
U3	1009	431	219	102	85	87	81
U4	623	267	123	54	86	79	69
U5	657	313	161	75	95	98	91
U6	607	289	143	65	95	94	85
				Mean R [%]	**94**	**92**	**86**
				Min	85	79	69
				Max	100	98	99

Dilution Linearity

Experiment:
Dilution linearity was assessed by serially diluting samples spiked with 600 pmol/l **recombinant** Vanin-1 with assay buffer.

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

Sample matrix	n	Recovery [%]					
		1+1		1+3		1+7	
		Mean	Range	Mean	Range	Mean	Range
Urine	6	94	91-97	91	88-93	80	78-82

Data showing dilution linearity of recombinant Vanin-1 in human urine samples:

Sample ID	iFGF23 [pg/ml]				Recovery [%]		
	Ref	1+1	1+3	1+7	1+1	1+3	1+7
U1	629	294	126	60	94	80	76
U2	564	297	141	62	105	100	87
U3	588	279	130	59	95	88	81
U4	609	286	140	63	94	92	82
U5	597	290	139	59	97	93	79
U6	642	293	146	62	91	91	78
				Mean R [%]	**94**	**91**	**80**
				Min	91	88	78
				Max	97	93	82

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 at different locations using different ELISA lots (in-between-run precision or reproducibility).

Within-Run Precision (Intra-Assay)

Experiment:
Two samples of known concentrations were tested three times within one kit lot by one operator.

Within-run (n=3)	Sample 1	Sample 2
Mean [pmol/l]	74	606
SD [pg/ml]	3	24
CV [%]	5	4

In-Between-Run Precision (Inter-Assay)

Experiment:
Two samples of known concentrations were tested nine times within two kit lots by two operators.

In-between run (n=9)	Sample 1	Sample 2
Mean (pmol/l)	77	605
SD (pmol/l)	5.1	21.1
CV (%)	7%	3%

DETECTION LIMIT & SENSITIVITY

To determine the sensitivity of the human Vanin-1 (urine) 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 standards containing recombinant Vanin-1, is diluted, measured five times and its concentration back calculated. The lowest dilution, which meets both criteria, is reported as the LLOQ.

The following values were determined for the human Vanin-1 (urine) ELISA:

LOD	**9.6 pmol/l**
LLOQ	**38 pmol/l**

SAMPLE STABILITY

Sample Collection and Storage

Urine is 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.

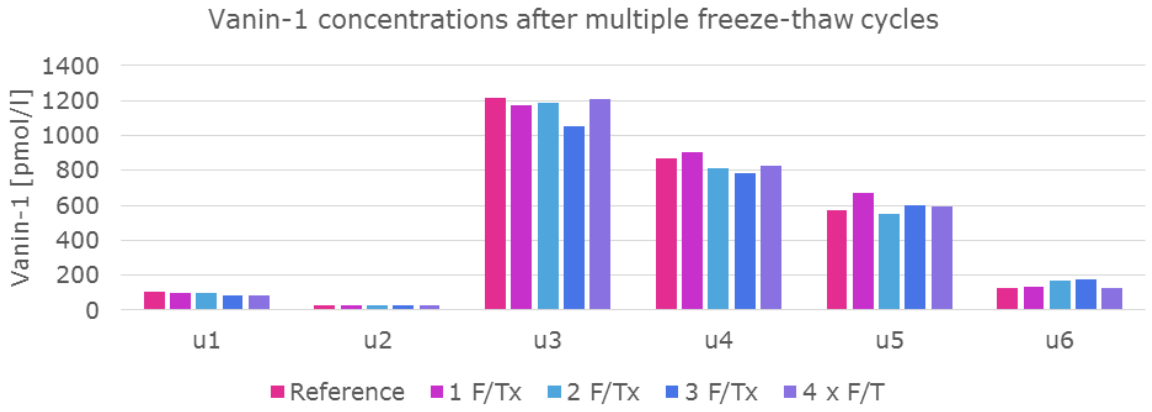
Urine
Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at -25°C or lower. Samples can undergo at least four freeze-thaw cycles.

Freeze-Thaw Stability of Samples Containing Endogenous Vanin-1

The stability of endogenous Vanin-1 was tested by comparing measurements in samples that had undergone four freeze-thaw cycles (F/T).

For freeze-thaw experiments, a set of human urine samples was aliquoted and freeze-thaw stressed. Samples can undergo at least four freeze-thaw cycles. The mean recovery of sample concentrations stressed by four freeze-thaw cycles is 96%.

Sample ID	Vanin-1 [pmol/l]					Recovery [%] 4 F/T cycles
	Ref	1x	2x	3x	4x	
U1	108	98	100	86	86	80
U2	29	26	26	29	29	100
U3	1218	1172	1186	1051	1210	99
U4	872	906	815	786	826	95
U5	569	670	552	603	593	104
U6	128	170	176	128	100	100
					Mean R [%]	**96**



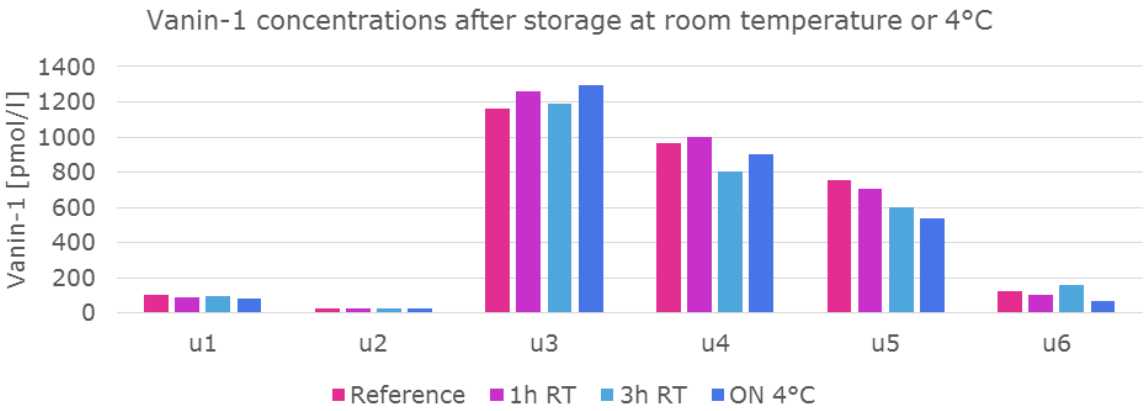
Benchtop Stability of Samples Containing Endogenous Vanin-1

The benchtop stability of endogenous Vanin-1 was tested by comparing Vanin-1 measurements in urine samples that had been stored at different temperatures.

For the assessment of the benchtop stability, a set of human urine samples was aliquoted and stored at -25°C, at room temperature or at 4°C. Samples can be stored for at least three hours at room temperature as well as overnight at 4°C . The mean recovery of sample concentrations after three hours at room temperature is 99%. The mean recovery of sample concentrations after overnight storage at 4°C is 87%.

Vanin-1 concentrations of samples stored at -25°C (reference), at room temperature (RT) or overnight (ON) at 4°C:

Sample ID	Vanin-1 [pmol/l]				Recovery [%] vs ref		
	Ref	1 h RT	3 h RT	ON 4°C	1 h RT	3 h RT	ON 4°C
U1	99	92	97	84	92	97	85
U2	25	26	27	27	102	107	107
U3	1158	1259	1191	1297	109	103	112
U4	963	1000	806	899	104	84	93
U5	752	707	600	536	94	80	71
U6	126	100	156	67	80	124	53
				Mean R [%]	**97**	**99**	**87**



SPECIFICITY

This assay recognizes endogenous and recombinant human Vanin-1.
The assay is potentially cross-reactive with Vanin-1 from various monkey species. No cross-reactivity with mouse Vanin-1.

The **specificity** of an ELISA is defined as its ability to exclusively recognize the analyte of interest. The specificity of the human Vanin-1 (urine) ELISA was shown by characterizing both the capture and the detection antibody though epitope mapping.
In addition, antibody affinities to Vanin-1 were tested by biolayer interferometry measurements (Octet), which measures the binding of antibodies to a Vanin-1-coated sensor. Both antibodies used in the human Vanin-1 (urine) ELISA bind to Vanin-1 with high affinity. Moreover, the specificity of the ELISA was established through competition experiments, which measure the ability of the antibodies to exclusively bind to Vanin-1.

Epitope Mapping

Antibody binding sites were determined by epitope mapping using microarray analysis (Pepperprint GmbH). The capture antibody binds to a single linear epitope located in the nitrilase domain of Vanin-1. The detection antibody binds to several epitopes distributed over the entire Vanin-1 molecule. For more information please contact info@bmgrp.com.

Competition of Signal

Competition experiments were carried out by pre-incubating human urine samples containing endogenous levels of Vanin-1 with an excess of capture antibody (CAB).
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 100%.

ID	Vanin-1 [pmol/l]		Recovery [%] Competition
	Reference	Reference + CAB	
U1	1253	29	98
U2	980	0	100
U3	107	0	100
U4	385	0	100
U5	869	0	100
U6	238	0	100
		Mean Comp. [%]	**100**

CALIBRATION

The human Vanin-1 (urine) immunoassay is calibrated against full-length human Vanin-1 protein (Uniprot ID: O95497 (https://www.uniprot.org/uniprot/O95497)).

REFERENCES & DOCUMENTS

Validation Guidelines

The assay is fully validated according to:

1. ICH Topic Q2 (R1) „Validation of Analytical Procedures: Text and Methodology"
2. EMEA/CHMP/EWP/192217/2009 Guideline on bioanalytical method validation
3. Bioanalytical Method Validation, Guidance for Industry, FDA, May 2018

Additional Documents Available Online (www.bmgrp.com)

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