VANIN-1 MOUSE/RAT

(EN) 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

rev.no. 191009

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CONTENT

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1) INTRODUCTION

Vanin- (VAN1) is a GPI-anchored glycoprotein of 513 amino acids consisting of a base domain and an enzymatic nitrilase domain (Boersma et al., 2014). The ectoenzyme catalyzes the hydrolysis of pantetheine to pantothenic acid (vitamin B5) and cyteamine and thus, is involved in the regulation of oxidative stress and inflammation (Maras et al., 1999). Vanin-1 has a broad tissue expression with the highest levels being observed in kidney tubular epithelial cells (Pitari et al., 2000). The GPI anchor of Vanin-1 can be cleaved by a yet unknown mechanism, resulting in Vanin-1 being shed into the extracellular space.
Function: Vanin-1 is an epithelial ectoenzyme activating the conversion of pantetheine into pantothenic acid (vitamin B5) and cysteamine (Pitari et al., 2000). It has been suggested that the release of cysteamine by Vanin-1 promotes oxidative tissue damage and inflammation by inhibiting the activity of antioxidants like superoxide dismutase (SOD) and glutathione (GSH) (Hosohata et al., 2011; Saghaei et al., 2012). Indeed, Vanin-1 knockout mice have elevated stores of GSH and are more resistant to oxidative injury induced by whole-body gamma irradiation (Berruyer et al., 2004). On the other hand, several reports indicate that Vanin-1 might also act as tissue sensor for oxidative stress. In mice, antioxidant response-like elements could be identified in the promotor region of Vanin-1, which enhance the expression of Vanin-1 in the presence of oxidative stress (Berruyer et al., 2004). Similarly, Vanin-1 expression was shown to be upregulated in a human proximal tubular cell line after exposure to organic solvents (Hosohata et al., 2011). After renal ischemia-reperfusion in rats, a model involving oxidative tissue damage, renal Vanin-1 expression was also found to be upregulated (Yoshida et al., 2002). The highest levels of Vanin-1 expression could be assigned to renal tubular epithelial cells, while no expression is detectable in glomeruli (Hosohata et al., 2011; Pitari et al., 2000). Hence, Vanin-1 released from renal cells could be detectable in urine. In a study aimed to identify biomarkers for renal tubular injury, Hosohata and colleagues could indeed show in a rat model of nephrotoxicant-induced injury that Vanin-1 is upregulated in renal tubules earlier than other markers and shed into urine (Hosohata et al., 2011). Subsequent studies further verified the validity of Vanin-1 as an early biomarker of renal tubular damage in drug-induced acute kidney injury (Hosohata et al., 2012, 2016a), obstructive nephropathy (Washino et al., 2019) and hydronephrosis (Hosohata et al., 2018), diabetic nephropathy (Fugmann et al., 2011), renal injury in experimental colitis (Hosohata et al., 2014) and spontaneously hypertensive rats under high salt intake (Hosohata et al., 2016b; Washino et al., 2018). Of note, Vanin-1 seems to have superior predictive value for acute kidney injury than established markers KIM-1, NGAL, or NAG (Fugmann et al., 2011; Hosohata, 2016; Hosohata et al., 2011).

Areas of interest:

- Acute kidney injury (Hosohata et al., 2016a)
- Diabetic nephropathy (Fugmann et al., 2011)
- Drug-induced acute kidney injury (Hosohata et al., 2016a)
- Hydronephrosis (Hosohata et al., 2018), obstructive nephropathy (Washino et al., 2019)

2) CONTENTS OF THE KIT

CONT	KIT COMPONENTS	QUANTITY
PLATE	Detachable microtiter strips pre-coated with anti-mouse Vanin-1 antibody	12 x 8 tests
WASHBUF	Wash buffer concentrate 20x, natural cap	1 x 50 ml
ASYBUF	Assay buffer, red cap, ready to use	1 x 7 ml
STOCK STD	Stock standard containing 200 pmol/l of recombinant mouse Vanin-1, red cap, lyophilised	1 vial
CTRL	Control, yellow cap, lyophilised (exact concentration after reconstitution see label)	1 vial
CONJ	Polyclonal sheep anti-mouse Vanin-1 antibody-HRPO, amber cap, ready to use	1 x 6ml
SUB	Substrate (TMB solution), blue cap, ready to use	1 x 13 ml
STOP	Stop solution, white cap, ready to use	1 x 7 ml

3) ADDITIONAL MATERIAL IN THE KIT

- 2 self-adhesive plastic films
- Quality control protocol
- Protocol sheet
- Instruction for use

4) MATERIAL AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 5 µl, 50 µl, 100 µl, and 1000 µl and disposable tips
- Distilled or deionised water
- Plate washer is recommended for washing, alternative multichannel pipette or manifold dispenser
- Refrigerator with 4°C (2-8°C)
- ELISA reader capable of measuring absorbance at 450 nm (with correction wavelength at 630 nm)
- Graph paper or software for calculation of results

5) REAGENTS AND SAMPLE PREPARATION

All reagents as supplied in the kit are stable at 4°C (2-8°C) until expiry date stated on the label of each reagent. Bring all reagents to room temperature before use.
Reconstituted STOCK STD and CTRL are stable at -25°C or lower until expiry date on label. STOCK STD and CTRL can undergo at least four freeze-thaw cycles.

Serum, plasma and urine samples are suitable for use in this assay. Do not change sample type during studies.. Lipemic or haemolysed samples may give erroneous results. We recommend duplicate measurements for all samples, standards and controls.

A. 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 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.
Before use in the assay bring samples to room temperature and mix samples gently to ensure the samples are homogenous. Samples with values above STD7 (200 pmol/l) can be diluted with ASYBUF (assay buffer).

Mouse samples

Mouse samples must be diluted 1+7 with assay buffer (ASYBUF), eg. 5 µl sample + 35 µl ASYBUF.
Diluted samples are stable at 4°C (2-8°C) overnight. Thus dilutions can be prepared one day before analysis.

Rat samples

Rat samples are used undiluted.

B. Preparation of CTRL (control):

Reconstitute the CTRL (control) in 200 µl distilled or deionised water . Leave at room temperature (18-26°C) for 10 min and vortex gently prior to use. The exact concentration is stated on the label.

C. Preparation of STOCK STD (stock standard):

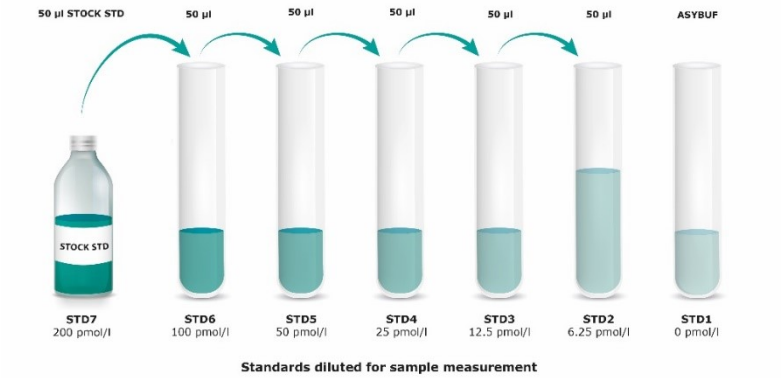
Reconstitute the mouse/rat Vanin-1 STOCK STD (stock standard) in 200 µl distilled water. Leave at room temperature (18-26°C) for 10 min and vortex gently prior to use.

D. Preparation of the standard curve:

1)	Use polypropylene tubes and mark them as STD6 to STD1 (see Graph 1).
2)	Mark STOCK STD as STD7.
3)	Pipette 50 µl of ASYBUF (assay buffer) into each tube marked as STD6 to STD1.
4)	Prepare a two-fold serial dilution to obtain STD6 to STD2: Pipette 50 µl of the reconstituted STOCK STD = STD7 into the tube labelled STD6. Mix thoroughly. Continue serial dilutions for STD5, STD4, STD3, STD2.
5)	ASYBUF serves as the zero standard (=STD1, 0 pmol/l).

Note: Always mix each tube thoroughly before the next step!

Graph 1: Preparation of STD7 to STD1

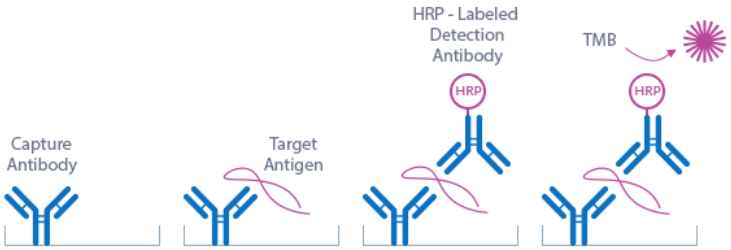


Preparation of WASHBUF (wash buffer):

Dilute the concentrate 1:20 (e.g. 50 ml WASHBUF + 950 ml distilled water). Crystals in the buffer concentrate will dissolve at room temperature (18-26°C). The undiluted WASHBUF is stable at 4°C (2-8°C) until expiry date stated on label. The diluted WASHBUF is stable up to one month at 4°C (2-8°C). Only use diluted WASHBUF when performing the assay.

6) PRINCIPLE OF THE ASSAY

The Vanin-1 mouse/rat ELISA kit is a sandwich enzyme immunoassay for the quantitative determination of mouse/rat Vanin-1 in serum, plasma or urine 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

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 .

7) ASSAY PROTOCOL

All reagents and samples must be at room temperature (18-26°C) before use in the assay.
Mark position for STD/CTRL/SAMPLE (Standard/Control/Sample) on the protocol sheet.
Take microtiter strips out of the aluminium bag. Store unused strips with desiccant at 4°C (2-8°C) in the aluminium bag. Strips are stable until expiry date stated on the label.

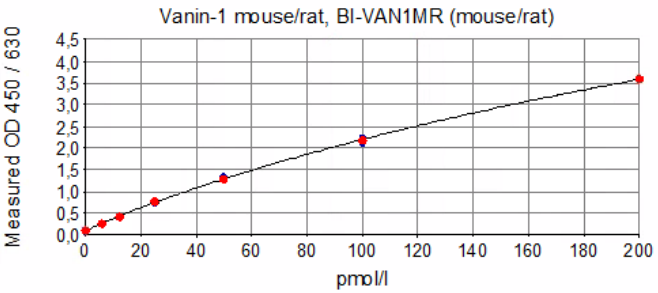
1)	Pipette 50 µl ASYBUF (assay buffer, red cap) into each well.
2)	Pipette 5 µl STD/CTRL/SAMPLE (standard/control/sample) into respective wells. ***Use mouse samples 1+7 pre-diluted. Use rat samples undiluted.***
3)	Add 50 µl CONJ (conjugate, amber cap) into each well. Swirl gently.
4)	**Cover tightly and incubate for 4 hours at room temperature (18-26°C), in the dark.**
5)	Aspirate and wash wells 5x with 300 µl diluted WASHBUF (wash buffer, natural cap). Remove remaining WASHBUF by strongly tapping plate against paper towel after the last wash.
6)	Add 100 µl SUB (substrate, blue cap) into each well. Swirl gently.
7)	**Cover tightly and incubate for 30 min at room temperature (18-26°C) in the dark.**
8)	Add 50 µl STOP (stop solution, white cap) into each well. Swirl gently.
9)	Measure absorbance immediately at 450 nm with reference 630 nm, if available.

8) CALCULATION OF RESULTS

Read the optical density (OD) of all wells on a plate reader using 450 nm wavelength (reference wavelength 630 nm). Construct a standard curve from the absorbance read-outs of the standards using commercially available software capable of generating a four-parameter logistic (4-PL) fit. Alternatively, plot the standards' concentration on the x-axis against the mean absorbance for each standard on the y-axis and draw a best fit curve through the points on the graph. Curve fitting algorithms other than 4-PL have not been validated and will need to be evaluated by the user.

Obtain sample concentrations from the standard curve. If required, pmol/l can be converted into pg/ml by applying a conversion factor (1 pg/ml = 19.2 pmol/l; Vanin-1 mouse/rat MW: 52.07 kDa). Respective dilution factors have to be considered when calculating the final concentration of the sample.

Example typical STD-curve:



The quality control (QC) protocol supplied with the kit shows the results of the final release QC for each kit lot. Data for OD obtained by customers may differ due to various influences and/or due to the normal decrease of signal intensity during shelf life. However, this does not affect validity of results as long as an OD of 1.00 or more is obtained for the STD with the highest concentration and the value of the CTRL is in range (target range see label).

9) ASSAY CHARACTERISTICS

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)			
Precision		**n**	**Average % CV**	
	Within-run	6	≤8	
	In-between-run	5	≤8	
Accuracy		**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 **mouse/rat 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	

For further information on assay and sample characteristics please visit our website www.bmgrp.com or contact our customer service by e-mail info@bmgrp.com or by phone +43/ 1/ 29107-45.

10) PRECISION

Within-run / intra-assay precision was assessed by measuring 2 samples of known concentrations 6 times within 1 Vanin-1 Mouse/Rat ELISA kit lot by 1 operator.

In-between-run /intra-assay precision was assessed by measuring 2 samples 5 times within 2 Vanin-1 Mouse/Rat ELISA kit lots by 2 different operators.

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

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

Detailed information on the Vanin-1 Mouse/Rat ELISA, e.g. assay performance characteristics, matrix comparisons, and stability data is available on our website www.bmgrp.com .

11) TECHNICAL HINTS

- Do not mix or substitute reagents with those from other lots or sources.
- Do not mix stoppers and caps from different reagents or use reagents between lots.
- Do not use reagents beyond expiration date.
- Protect reagents from direct sunlight.
- Substrate solution should remain colourless until added to the plate.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.

12) PRECAUTIONS

All liquid reagents contain ≤ 0.1% Proclin 950 as preservative. Avoid contact with skin and mucous membrane. Proclin 950 is not toxic in concentrations used in this kit. It may cause allergic skin reactions – avoid contact with skin or eyes.

- Do not pipette by mouth.
- Do not eat, drink, smoke, or apply cosmetics where reagents are used.
- Wear gloves, glasses, and lab coat while performing this assay.
- Sulfuric acid is irritating to the eyes and skin. Avoid contact with skin and mucous. Irritations are possible. Flush with water if contact occurs.

13) LITERATURE

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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

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