1) INTRODUCTION

Vanin-1 (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

CONTENTS	KIT COMPONENTS	QUANTITY
PLATE	Anti-human Vanin-1 antibody precoated microtiter strips in stripholder packed in aluminium bag with desiccant	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
STD	Standards 1-7 (0; 37.5; 75; 150; 300; 600; 1200 pmol/l), recombinant human Vanin-1 protein in buffer, white caps, lyophilised	7 vials
CTRL	Controls A + B, yellow caps, lyophilised (exact concentrations after reconstitution see labels)	2 vials
CONJ	Conjugate, (polyclonal sheep anti-human Vanin-1 antibody-HRPO), amber bottle, amber cap, ready to use	1 x 7 ml
SUB	Substrate (TMB solution), amber bottle, 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 10 µl, 50 µl, 100 µl, 300 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.

Sample preparation:
Urine samples are suitable for use in this assay. For longer storage aliquot samples and store at -25°C or lower. Samples are stable for at least four freeze-thaw cycles. Spin down samples to avoid erroneous results.
We recommend duplicates for all values. The results should be reported as a normalized ratio to urinary creatinine concentration to control for variations in urine flow rate.
If samples read higher than STD7, we recommend to dilute samples with ASYBUF and test again.

For further information on sample stability please visit our website www.bmgrp.com (see Validation Data) or contact our customer service by e-mail info@bmgrp.com or by phone +43/ 1/ 29107-45.

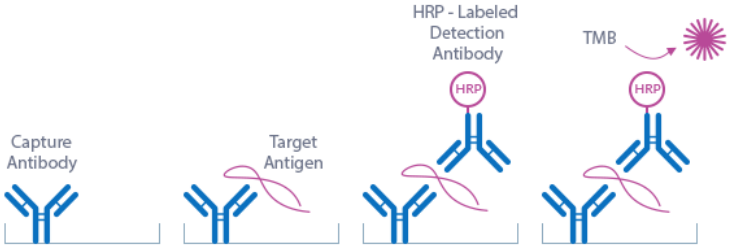
Reconstitution/Handling:

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. 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.

STD (Standards) + CTRL (Controls): Pipette 200 µl of distilled or deionised water into each vial. Leave at room temperature (18-26°C) for 10 min. Vortex gently. The exact concentration is printed on the label. Reconstituted STDs and CTRLs are stable for 3 h at room temperature (18-26°C) or at -25°C or lower until expiry date stated on the label. STDs and CTRLs are stable for four freeze-thaw cycles.

6) 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 samples.
In a first step, assay buffer is pipetted into the wells of the microtiter strips. Thereafter, standard/control/sample and detection antibody (CONJ, 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.



7) ASSAY PROTOCOL

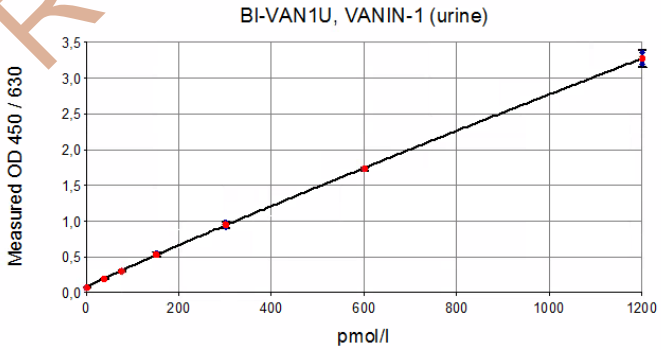
All reagents and samples must be at room temperature (18-26°C) before use in the assay.
Mark position for STD/CTRL/SAMPLE (standard/control/sample) on the protocol sheet.
Take microtiter strips out of the aluminium bag. Store unused strips with desiccant at 4°C (2-8°C) in the aluminium bag. Strips are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

1. Pipette 50 µl ASYBUF (assay buffer, red cap) into each well.
2. Pipette 10 µl STD/CTRL/SAMPLE (standard/control/sample) into respective wells. Swirl gently.
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 (correction wavelength 630 nm). Construct the standard curve from the OD values of the STD. Use commercially available software or graph paper. Obtain sample concentration from this standard curve. The assay was evaluated with logit-log and 4PL algorithm curve fitting. Different curve fitting methods need to be evaluated by the user. 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 1.50 or more is obtained for the STD with the highest concentration and the values of the CTRLs are in range (target ranges see labels).

9) ASSAY CHARACTERISTICS

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		
Accuracy		**n**	**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. The VNN1 gene is conserved in chimpanzee, rhesus monkey, dog, cow, mouse, rat, and chicken https://www.ncbi.nlm.nih.gov/homologene/32130. Cross-reactivity has not been tested in non-human samples. A specific Vanin-1 mouse/rat kit is separately available (cat.nr. BI-VAN1MR).				
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	1,131		

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

10) PRECISION

Within-run precision (Intra-assay): Two samples of known concentrations were tested three times within one kit lot by one operator.

In-between-run precision (Inter-assay): Two samples of known concentrations were tested nine times within two kit lots by two operators.

Intra-assay (n= 3)	Sample 1	Sample 2
Mean (pmol/l)	74	606
SD (pmol/l)	3	24
CV (%)	5	4

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

Detailed information on the Vanin-1 urine ELISA, e.g. assay performance characteristics and stability data, is available on our website www.bmgrp.com (see Validation Data).

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 test components of human source were tested against HIV-Ab, HCV-Ab and HBsAg and were found negative. Nevertheless, they should be handled and disposed as if they were infectious.
All liquid reagents contain ≤ 0.1% Proclin 950 as preservative. 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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SYMBOLS

Expiry date / Verfallsdatum / Date de péremption / Data di scadenza /Fecha de caducidad / Data de validade / Uiterste gebruiksdatum / Udløbsdato / Utgångsdatum / Termin Ważności / Lejárati idő / Doba exspirácie / Doba exspirace

Consider instructions for use / Bitte Gebrauchsanweisung beachten / Consultez la notice d'utilisation / Consultare le istruzioni per l'uso / Consulte las instrucciones de utilización / Consulte as instruções de utilização / Raadpleeg de gebruiksaanwijzing / Se brugsanvisningen / Läs anvisningarna före användning / Proszę przeczytać instrukcję wykonania / Vegyük figyelembe a használati utasításban foglaltakat / Postupujte podľa pokynov na použitie / Postupujte dle návodu k použití

Lot-Batch Number / Charge-Chargennummer / Lot-Code du lot / Lotto-Numero di lotto / Lote-Código de lote / Lote-Código do lote / Lot-Partijnummer / Lot-Batchkode / Lot-Satskod / Numer serii / Lot-Batch szám / Číslo šarže / Číslo šarže

Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por / Vervaardigd door / Fabrikation af / Tillverkad av / Wyprodukowane pr / Gyártotta / Vyrobené / Vyrobeno

Catalogue Number / Bestellnummer / Numéro de référence / Numero di riferimento / Número de referencia / Número de referência / Referentienummer / Referencenummer / Katalognummer / Numer katalogowy / Katalógusszám / Katalógové číslo / Katalogové číslo

Store at between / Lagerung bei zwischen / Conserver à entre / Conservare a tra / Conservar a temp. entre / Armazene a entre / Bewaar bij tussen / Opbevares mellem / Förvaras vid / Przechowywać w / Tároljuk …… között / Skladujte v rozsahu / Skladujte v rozmezí

Contains sufficient for x tests / Inhalt ausreichend für x Tests / Contient suffisant pour x tests / Contenuto sufficiente per x test / Contiene suficiente para x pruebas / Contém suficiente para x testes / Bevat voldoende voor x bepalingen / Indeholder tilstrækkeligt til x prøver / Innehållet räcker till x analyser / Zawartość na x testów / Tartalma X teszt elvégzésére elegendő / Obsahuje materiál pre x testov / Obsahuje materiál pro x testů

BI- VAN1U VANIN-1 (urine) ELISA
ASSAY PROTOCOL AND CHECKLIST

PREPARATION OF REAGENTS:

- ❑ Bring all reagents to room temperature (18-26°C).
- ❑ Prepare reagents and samples as instructed.
- ❑ Bring unused and prepared components to the storage temperature mentioned in the package insert.
- ❑ Take microtiter strips out of the alu bag and mark positions on the protocol sheet.

TEST PROCEDURE:

- ❑ Step 1) Pipette 50 µl ASYBUF (assay buffer, red cap) into each well.
- ❑ Step 2) Pipette 10 µl STD/CTRL/SAMPLE (Standard/Control/Sample) into respective wells.
- ❑ Step 3) Add 50 µl CONJ (Conjugate, amber cap) into each well. Swirl gently.
- ❑ **Step 4) Cover tightly and incubate for 4 hours at room temperature (18-26°C).**
- ❑ Step 5) Aspirate and wash wells with 300 µl WASHBUF (Wash buffer, natural cap) five times. Remove remaining buffer by strongly tapping plate against paper towel.
- ❑ Step 5) Add 100 µl SUB (Substrate, blue cap) into each well. Swirl gently.
- ❑ **Step 6) Incubate for 30 minutes at room temperature (18-26°C) in the dark.**
- ❑ Step 7) Add 50 µl STOP (Stop solution, white cap) into each well. Swirl gently.
- ❑ Step 8) Measure absorbance immediately at 450 nm with reference 630 nm, if available.