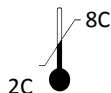


IMUBIND® vWF ELISA

REF 828



INTENDED USE

The IMUBIND® vWF ELISA is an enzyme-linked immunoassay for the measurement of vWF antigen in human plasma. The assay is for research use only. It is not intended for diagnostic or therapeutic procedures.

EXPLANATION OF THE TEST

Von Willebrand Factor (vWF) is a large, multimeric protein (molecular weight of 1,000-20,000 kD) composed of repeating 270 kD subunits containing 2050 amino acid residues. vWF is synthesized by endothelial cells and megakaryocytes, and is present in multimeric form in the basement membrane of the subendothelium, in plasma and platelets. The half-life of vWF in plasma is approximately 20 hours. Degraded forms of vWF are excreted in urine.^{1,2} vWF functions as a carrier protein for Factor VIII, the coagulation protein absent in haemophilia A. It promotes platelet adhesion to damaged endothelium and participates in the platelet to platelet cohesion necessary for thrombus formation. Together with fibronectin and collagen, vWF functions in maintaining vessel wall integrity. Since vWF is synthesized in endothelial cells, it has been used as a marker for endothelial cell function and integrity. Measurements of vWF have been applied in a large number of basic investigations on endothelial cell function.

Patients with severe von Willebrand disorder (vWD), classified as type III, suffer from a complete absence of vWF in their plasma and urine. Patients with decreased circulating levels of vWF suffer from milder forms of the disorder, classified as type I, type IIa and type IIb.^{2,3} vWD type I is a common disorder⁴.

Studies have reported that in large vessel lesions of the endothelium such as arteriosclerosis, vWF levels have increased. However, in other studies, no significant increase of the factor has been observed.⁵ Increased levels of the vWF/Factor VIII complex have also been reported in postoperative patients and patients with thrombosis.⁶

vWF levels were measured in plasma and urine samples from patients with Type I diabetes mellitus (insulin-dependent) with and without signs of microangiopathy (retinopathy and nephropathy).⁵ Plasma vWF levels were significantly higher in all groups of patients compared to control patients, while urinary vWF levels were significantly higher in patients with microangiopathy. Qualitative differences in the excreted forms of vWF were observed only in those patients with clinical signs of nephropathy, where there was a shift towards excretion of the high molecular weight fragments.

PRINCIPLE OF THE PROCEDURE

The IMUBIND vWF ELISA is a "sandwich" ELISA using a goat polyclonal antibody as the capture antibody. Samples incubate in precoated micro-test wells and the same polyclonal antibody, horseradish peroxidase (HRP) conjugated, is used to detect the bound vWF antigen. The addition of perborate/3,3',5,5' - tetramethylbenzidine (TMB) substrate, and its subsequent reaction with the HRP, creates a blue colored solution. Sensitivity is enhanced by the addition of a 0.5M sulfuric acid stop solution, yielding a yellow colored solution. vWF levels are determined by measuring and comparing the absorbance of sample solutions at 450 nm against those of a standard curve developed using calibrated antigen.

REAGENTS

96 Anti-Human vWF IgG Coated Microwells with an acetate cover sheet
6 vials of vWF standards, 0 - 10 mU/mL (lyophilized)
1 vial of Detection Antibody, HRP-conjugated anti-human vWF (135 µL)
1 vial of Detection Antibody Diluent (lyophilized)
1 vial of Substrate, TMB (11 mL)
1 packet of Wash Buffer, PBS with 0.05% Tween 20, pH 7.4

There are sufficient reagents to assay 42 plasma samples and generate a 6-point standard curve (both tested in duplicate). Samples may be patient, control or reference plasmas.

WARNINGS AND PRECAUTIONS

For Research Use Only.

Source material for some of the reagents in this kit is of human origin. This material has been found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1, HIV-2) using FDA approved methods. As no known test method provides complete assurance that products derived from human blood will not transmit HBsAg, HCV, HIV-1, HIV-2 or other blood-borne pathogens, reagents should be handled as recommended for any potentially infectious human specimen. Discard all waste associated with test specimens and human source reagents in a biohazard waste container.

Limited for research use only in the United States. For *in vitro* use only. Not for internal use in humans or animals. Do not use the kit components beyond the stated expiration date. Do not mix reagents from different kits. Avoid microbial contamination of the reagents. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled. Do not pipette reagents by mouth.

vWF standards	Danger		H317, H334, P280, P302 + P352, P362		
Detection antibody	Warning		H319, P264, P280, P305+P351+P338, P337+P313		
Detection Antibody Diluent	Danger	  	<table><tr><td>CONT</td><td>Polyethylene glycol octylphenol ether</td></tr></table> H318, H412, P280, P273, P305+P351+P338	CONT	Polyethylene glycol octylphenol ether
CONT	Polyethylene glycol octylphenol ether				
PBS-Tween packet	Warning		H319, P264, P280, P305+P351+P338, P337+P313		

Hazard Statements:	H317	May cause an allergic skin reaction.
	H318	Causes serious eye damage.
Precautionary Statements:	H319	Causes serious eye irritation.
	H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled
	H412	Harmful to aquatic life with long lasting effects.
	P264	Wash thoroughly after handling.
	P273	Avoid release to the environment.
	P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
	P302 + P352	IF ON SKIN: Wash with plenty of water.
	P305 + P351+ P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P337 + P313	If eye irritation persists: Get medical advice/attention.
	P362	Take off contaminated clothing and wash before reuse.

REAGENT PREPARATION AND STORAGE

Unopened and lyophilized reagents are stable until the expiration date printed on the box when stored as instructed.

1. Anti-Human vWF IgG Coated Microwells

Once removed from the foil pouch, the microwells should be used within 30 minutes. Unused strips may be stored at 2°-8°C for 4 weeks when sealed in the original pouch with the desiccant present, protected from any moisture.

2. Standards

1. Add 1.0 mL filtered deionized water to the 0.5, 1.0, 2.0, 5.0 and 10.0 mU/mL standard vials.
2. Add 2.0 mL filtered deionized water to the 0 mU/mL standard vial.
3. Agitate gently. Do not shake!

Reconstituted vWF standards are stable for up to 1 month when stored at -20°C or colder.

3. Detection Antibody

Supplied as a concentrated solution. Detection Antibody is diluted to working strength immediately before adding to the microwells. Concentrated Detection Antibody is stable until the expiration date stated on the vial when stored at 2°-8°C. Unused working strength Detection Antibody should be discarded.

4. Detection Antibody Diluent

1. Add 20 mL of filtered deionized water to the Detection Antibody Diluent vial.
2. Mix well.

Reconstituted Detection Antibody Diluent may be used for up to 1 month when stored at 2°-8°C.

5. Substrate

Supplied ready to use. Once opened, the substrate may be used for up to 1 month when stored at 2°-8°C.

6. Wash Buffer

1. Dissolve the contents of the Wash Buffer packet in 900 mL filtered deionized water.
2. QS. to a final volume of 1 Liter with filtered deionized water.
3. Mix well and confirm pH is 7.4 (adjust if necessary).

Wash Buffer may be used for up to 1 month when stored at 2°-8°C.

7. Sample Buffer

Prepare an appropriate amount of Sample Buffer by adding BSA to Wash Buffer to a final concentration of 3% w/v (3 gm BSA/100 mL Wash Buffer). Sample Buffer may be used for up to 2 weeks when stored at 2°-8°C.

SPECIMEN COLLECTION AND PREPARATION

Either citrate or EDTA collected platelet poor plasma may be used for this assay. See "Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline-Fifth Edition", CLSI Document H21-A5, Vol. 28, No.5, 2008. Plasma collection should be performed as follows:

1. Collect 9 parts of blood into 1 part of 3.2% (0.109 M) trisodium citrate anticoagulant solution.
2. Centrifuge the blood sample at 1,500 x g for 15 minutes.
3. Plasma should be stored at 2°-8°C and assayed within 4 hours. Alternatively, plasma may be stored at -20°C for up to 6 months.
4. Frozen plasma should be thawed rapidly at 37°C. Thawed plasmas should be stored at 2°-8°C and assayed within 4 hours.
5. Dilute plasma sample 1:100 in Sample Buffer.

PROCEDURE

Materials Provided – See Reagents

Material Required But Not Provided

0.22 µm filtered deionized H₂O
50-300 µL eight channel multi-pipette
0-200 µL, 200-1000 µL single pipettes
microwell plate reader for reading absorbance at 450 nm
microwell plate washer (optional)
0.5 M H₂SO₄ **Caution:** Handle Sulphuric acid with great care. Avoid any skin and eye contact. Wear protection glasses and gloves when handling.
Bovine Serum Albumin

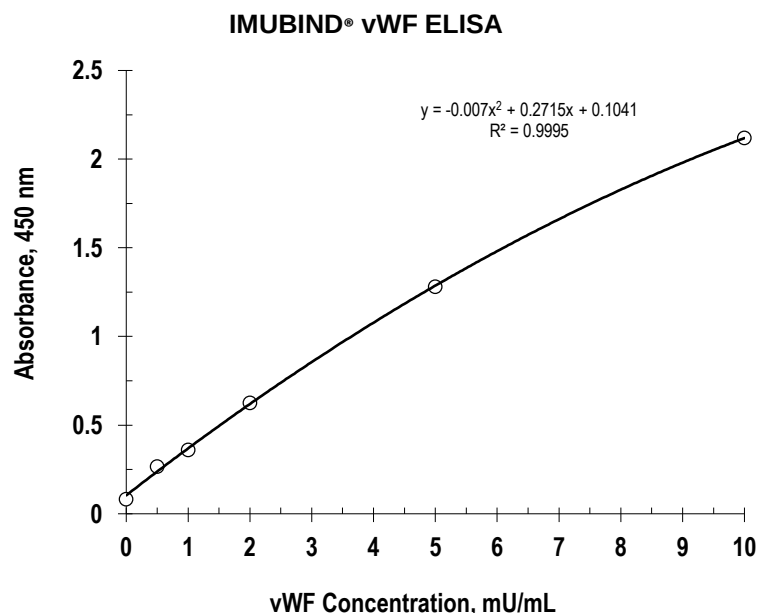
Assay Procedure

1. Open the foil pouch and remove the microwells. Remove those microwells that will not be used and replace them in the foil pouch with the desiccant inside. Reseal the pouch and store at 2°-8°C.
2. Add 100 µL of vWF standard or diluted sample to a microwell, cover with the acetate sheet and incubate for 1 hour at room temperature. It is recommended to perform measurements in duplicate.
3. Wash the microwells 4 times with wash buffer (250 µL per well).
4. Prepare working strength Detection Antibody by diluting the concentrate 1:100 as follows: add 20 µL of Detection Antibody to 2 mL of Detection Antibody Diluent. Then add 100 µL of working strength Detection Antibody to each microwell, cover with the acetate sheet and incubate for 1 hour at room temperature.
5. Wash the microwells 4 times with wash buffer (250 µL per well).
6. Add 100 µL of Substrate to each microwell, cover with acetate sheet and incubate for 20 minutes at room temperature. A blue color will develop.
7. Stop the enzymatic reaction by adding 50 µL of 0.5M H₂SO₄. Tap the sides of the strip wells to ensure even distribution of the H₂SO₄. The solution color will turn yellow. Immediately read the absorbances of the solutions on a microwell plate reader set at a wavelength of 450 nm.

RESULTS

Construct a standard curve by plotting the mean absorbance value for each vWF Standard versus its corresponding concentration. A standard curve should be generated each time the assay is performed. The following standard curve is for demonstration purposes only.

Representative Standard Curve



CALCULATION

Use the mean absorbance value for each diluted sample to interpolate its vWF concentration from the standard curve. Multiply the concentration determined from the standard curve by 100 (the dilution factor) to obtain the vWF concentration in the original plasma sample.

LIMITATIONS OF THE PROCEDURE

There are no known limitations for this assay.

BIBLIOGRAPHY

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3. Ruggeri, Z., M. *Thrombosis and Haemostasis*. Eds. Verstraate *et al.*, Leuven University Press, 1987: 419-445.
4. Rodeghiero, F., Castaman, G., Dini, E. *Blood* 1987; **69**: 454-459.
5. Silveira, A. Studies of vWF in urine and plasma in health and disease. Thesis. Karolinska Institute, Stockholm, 1990.
6. Bergman, A., Andréen, M., and Blombäck, M. *Acta Anaesthes Scand*. 1989.
7. *Lancet* (Editorial), May 28, 1988: 1203-1204.
8. Guistolisi, R., *et al.* *Lancet*, June 23, 1988: 227-228.
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DEFINITION OF SYMBOLS

	Consult instructions for use
	Manufacturer
	Refer to Safety Data Sheet
	Store at 2°C to 8°C
	Lot Number
	Expiration Date
	Catalog Number
	Contains sufficient for <n> tests
	Contains...