

INTENDED USE

This human von Willebrand Factor (vWF) antigen assay is intended for the quantitative determination of total vWF antigen (VWF:Ag) in human plasma.

For research use only.

BACKGROUND

vWF is a plasma glycoprotein that circulates as disulfide linked multimers ranging in size from 520 kDa dimers to greater than 20,000 kDa [1]. Each 260 kDa 2,050 amino acid monomer binds one Factor VIII molecule protecting against proteolytic degradation and clearance [2]. vWF also binds platelets and collagen and is required for platelet plug formation [3]. ADAMTS13 cleaves vWF at vascular injury sites, in circulation, and anchored on the endothelial surface [4]. Type 1 von Willebrand disease due to quantitative vWF deficiency is characterized by mild bruising and bleeding [5].

ASSAY PRINCIPLE

Human vWF will bind to the monoclonal capture antibody coated on the microtiter plate. After appropriate washing steps, biotinylated monoclonal anti human vWF primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody is reacted with peroxidase conjugated streptavidin. Following an additional washing step, TMB substrate is used for color development at 450nm. A standard calibration curve is prepared using dilutions of human vWF and is measured along with the test samples. Color development is proportional to the concentration of vWF in the samples.

STANDARD CALIBRATION

vWF standard provided is calibrated against the VWF:Ag value of the WHO 6th International Standard for Blood Coagulation Factor VIII and von Willebrand Factor in Plasma distributed by NIBSC (07/316), South Mimms, Potters Bar, Hertfordshire, UK.

Lot 117L: 1,000 ng = 0.117 IU

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-human vWF antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Human vWF standard:** 1 vial lyophilized standard
- **Anti-human vWF primary antibody:** 1 vial lyophilized polyclonal antibody
- **Horseradish peroxidase-conjugated streptavidin:** 1 vial concentrated HRP labeled streptavidin
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standard and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H₂SO₄ or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- TBS buffer:** 0.1M Tris, 0.15M NaCl, pH 7.4
- Blocking buffer (BB):** 3% BSA (w/v) in TBS
- 1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water

SAMPLE COLLECTION

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard

Reconstitute standard by adding 1ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 1000ng/ml standard solution.

Dilution table for preparation of human vWF standard:

vWF concentration (ng/ml)	Dilutions
500	500 μl (BB) + 500 μl (from vial)
250	500 μl (BB) + 500 μl (500ng/ml)
100	600 μl (BB) + 400 μl (250ng/ml)
50	500 μl (BB) + 500 μl (100ng/ml)
20	600 μl (BB) + 400 μl (50ng/ml)
10	500 μl (BB) + 500 μl (20 ng/ml)
5	500 μl (BB) + 500 μl (10ng/ml)
2	600 μl (BB) + 400 μl (5ng/ml)
1	500 μl (BB) + 500 μl (2ng/ml)
0.5	500 μl (BB) + 500 μl (1ng/ml)
0	500 μl (BB) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition

Remove microtiter plate from bag and add 100 μl vWF standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 μl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

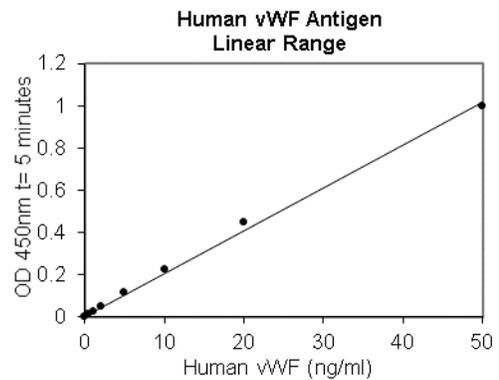
NOTE: The assay measures total human vWF in the 0.5-500 ng/ml range. Samples giving human vWF levels above 500ng/ml should be diluted in blocking buffer before use. A 1:200-1:1,000 dilution for normal human plasma is suggested for best results.

Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100 μl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 μl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Streptavidin-HRP Addition

Briefly centrifuge vial before opening. Dilute 2.5µl of HRP conjugated streptavidin into 2.5ml blocking buffer to generate a 1:1,000 dilution. Add 0.4ml of 1:1,000 dilution to 9.6ml of blocking buffer to generate a 1:25,000 dilution. Add 100µl of the 1:25,000 dilution to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.



Substrate Incubation

Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H₂SO₄ or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

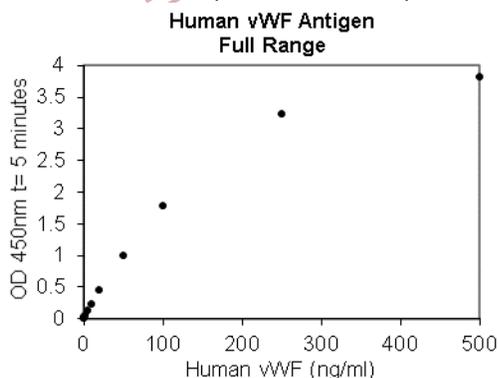
Measurement

Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A₄₅₀).

Calculation of Results

Plot A₄₅₀ against the amount of vWF in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of vWF in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):



EXPECTED VALUES

The concentration of vWF in normal human plasma is approximately 10.0 µg/ml [6]. The normal range is 0.5-2 IU/ml with values less than 0.5 IU/ml indicating type 1 von Willebrand disease [7].

PERFORMANCE CHARACTERISTICS

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD₄₅₀: 0.043-0.054) and calculating the corresponding concentration. The MDD was 0.27 ng/ml.

Intra-assay Precision: These studies are currently in progress. Please contact us for more information.

Inter-assay Precision: These studies are currently in progress. Please contact us for more information.

Recovery: These studies are currently in progress. Please contact us for more information.

Linearity: These studies are currently in progress. Please contact us for more information.

Sample Values: Samples were evaluated for the presence of the antigen at varying dilutions.

Sample Type	Dilution	Mean (µg/mL)
Citrate Plasma	1:200	12.6
	1:400	12.3
	1:800	12.2

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

REFERENCES

1. Stocksclaeder M, *et al.*: Blood Coagul Fibrinolysis. 2014, 25:206-216.
2. Vlot AJ, *et al.*: Blood 1996, 87:1809-1816.
3. Peyvandi F, *et al.*: Blood Transfus. 2011, 9:s3-s8.
4. Zheng XL: Annu Rev Med. 2015, 66:211-225.
5. Leebeek FWG and Eikenboom JCJ: N Engl J Med. 2016, 375:2067-2080.
6. Marder VJ, *et al.*: Hemostasis and Thrombosis: Basic Principles and Clinical Practice 2012, 203.
7. Ng C, *et al.*: Blood 2015, 125:2029-2037.

Example of ELISA Plate Layout

96 Well Plate: 22 Standard wells, 74 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	250 ng/ml	500 ng/ml	
B	0	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	250 ng/ml	500 ng/ml	
C												
D												
E												
F												
G												
H												