



# VWF (Human) ELISA Kit

Catalog Number KA0512

96 assays

Version: 37

Intended for research use only

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## Table of Contents

<b>Introduction .....</b>	<b>3</b>
Background .....	3
Principle of the Assay .....	3
<b>General Information .....</b>	<b>4</b>
Materials Supplied.....	4
Storage Instruction .....	4
Materials Required but Not Supplied .....	4
Precautions for Use .....	5
<b>Assay Protocol .....</b>	<b>6</b>
Reagent Preparation.....	6
Sample Preparation .....	7
Assay Procedure .....	7
<b>Data Analysis.....</b>	<b>9</b>
Calculation of Results.....	9
Performance Characteristics .....	10
<b>Resources.....</b>	<b>12</b>
Troubleshooting.....	12
References .....	13
Plate Layout .....	14

## **Introduction**

### **Background**

Von willebrand factor (VWF) is a multimeric glycoprotein that circulates in blood, forming a noncovalent complex with procoagulant factor VIII (1). During normal homeostasis, the larger multimers of VWF are responsible for facilitating platelet plug formation by forming a bridge between platelet glycoprotein IB and exposed collagen in the subendothelium (2-3). The congenital dysfunctional state of VWF causes a moderate to severe bleeding diathesis-von Willebrand disease (VWD).

### **Principle of the Assay**

The VWF (Human) ELISA kit is designed for detection of VWF in human plasma, serum, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human VWF in approximately 5 hours. A monoclonal antibody specific for human VWF has been pre-coated onto a 96-well microplate with removable strips. VWF in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for human VWF, which is recognized by a streptavidin-peroxidase (SP) conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

## General Information

### Materials Supplied

List of component

Component	Amount
Human VWF Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human VWF.	96 (8x12) wells
Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.	3 slices
Human VWF Standard: Human VWF in a buffered protein base (lyophilized, calibrated against WHO 2 <sup>nd</sup> International Standard).	84 mIU
Biotinylated Human VWF Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against human VWF.	120 µL
MIX Diluent Concentrate (10x): A 10-fold concentrated buffered protein base.	30 mL
Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant.	30 mL x 2
SP Conjugate (100x): A 100-fold concentrate.	80 µL
Chromogen Substrate (1x): A stabilized peroxidase chromogen substrate tetramethylbenzidine.	8 mL
Stop Solution (1x): A 0.5 N hydrochloric acid solution to stop the chromogen substrate reaction.	12 mL

### Storage Instruction

- ✓ Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- ✓ Store SP Conjugate and Biotinylated Antibody at -20°C.
- ✓ Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- ✓ Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- ✓ Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

### Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Pipettes (1-20 µL, 20-200 µL, 200-1000 µL and multiple channel).
- ✓ Deionized or distilled reagent grade water.

**Precautions for Use**

- ✓ This product is for Research Use Only and is not intended for used in diagnostic procedures.
- ✓ Prepare all reagents (diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.
- ✓ Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
- ✓ Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.
- ✓ The Stop Solution is an acidic solution.
- ✓ The kit should not be used beyond the expiration date.

## Assay Protocol

### Reagent Preparation

- ✓ Freshly dilute all reagents and bring all reagents to room temperature before use.
- ✓ MIX Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 10-fold with reagent grade water to produce a 1x solution. Store for up to 30 days at 2-8°C.
- ✓ Human VWF Standard: Reconstitute the Human VWF Standard (84 mIU) with 2.1 mL of MIX Diluent to generate a 40 mIU/mL standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (40 mIU/mL) 2-fold with equal volume of MIX Diluent to produce 20, 10, 5, 2.5, 1.25 and 0.625 mIU/mL solutions. MIX Diluent serves as the zero standard (0 mIU/mL). Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

Standard Point	Dilution	[VWF] (mIU/mL)
P1	1 part Standard (40 mIU/mL)	40
P2	1 part P1 + 1 part MIX Diluent	20
P3	1 part P2 + 1 part MIX Diluent	10
P4	1 part P3 + 1 part MIX Diluent	5.0
P5	1 part P4 + 1 part MIX Diluent	2.5
P6	1 part P5 + 1 part MIX Diluent	1.25
P7	1 part P6 + 1 part MIX Diluent	0.625
P8	MIX Diluent	0.00

- ✓ Biotinylated Human VWF Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 50-fold with MIX Diluent to produce a 1x solution. The undiluted antibody should be stored at -20°C.
- ✓ Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 20-fold with reagent grade water to produce a 1x solution.
- ✓ SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with MIX Diluent to produce a 1x solution. The undiluted conjugate should be stored at -20°C.

## **Sample Preparation**

- ✓ Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect plasma. A 100-fold sample dilution is suggested into MIX Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- ✓ Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. A 100-fold sample dilution is suggested into MIX Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- ✓ Cell Culture Supernatant: Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris and collect supernatant. Samples can be stored at -80°C. Avoid repeated freeze-thaw cycles. *Applicable samples may also include biofluids, cell culture, and tissue homogenates. If necessary, user should determine optimal dilution factor depending on application needs.*

Refer to Dilution Guidelines for further instruction

Guidelines for Dilutions of 100-fold or Greater (for reference only; please follow the insert for specific dilution suggested)	
100x	10000x
A. 4 µL sample: 396 µL buffer (100X) = 100-fold dilution  Assuming the needed volume is less than or equal to 400 µL.	A. 4 µL sample: 396 µL buffer (100x) B. 4 µL of A: 396 µL buffer (100x) = 10000-fold dilution  Assuming the needed volume is less than or equal to 400 µL.
1000x	100000x
A. 4 µL sample: 396 µL buffer (100x) B. 24 µL of A: 216 µL buffer (10x) = 1000-fold dilution  Assuming the needed volume is less than or equal to 240 µL.	A. 4 µL sample: 396 µL buffer (100x) B. 4 µL of A: 396 µL buffer (100x) C. 24 µL of B: 216 µL buffer (10x) = 100000-fold dilution  Assuming the needed volume is less than or equal to 240 µL.

## **Assay Procedure**

1. Prepare all reagents, standards solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with

desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.

3. Add 50  $\mu$ L of Human VWF Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
4. Wash the microplate manually or automatically using a microplate washer. Invert the plate and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If washing manually, wash five times with 200  $\mu$ L of Wash Buffer per well. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a microplate washer, wash six times with 300  $\mu$ L of Wash Buffer per well; invert the plate and hit 4-5 times on absorbent material to completely remove the liquid.
5. Add 50  $\mu$ L of Biotinylated Human VWF Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours.
6. Wash the microplate as described above.
7. Add 50  $\mu$ L of SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
8. Wash the microplate as described above.
9. Add 50  $\mu$ L of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 15 minutes or until the optimal blue color density develops.
10. Add 50  $\mu$ L of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
11. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

✓ Assay Summary

1. Add 50  $\mu$ L of Standard or Sample per well. Incubate 2 hours.
2. Wash, then add 50  $\mu$ L of Biotinylated Antibody per well. Incubate 2 hours.
3. Wash, then add 50  $\mu$ L of SP Conjugate per well. Incubate 30 minutes.
4. Wash, then add 50  $\mu$ L of Chromogen Substrate per well. Incubate 15 minutes.
5. Add 50  $\mu$ L of Stop Solution per well. Read at 450 nm immediately.



## Data Analysis

### Calculation of Results

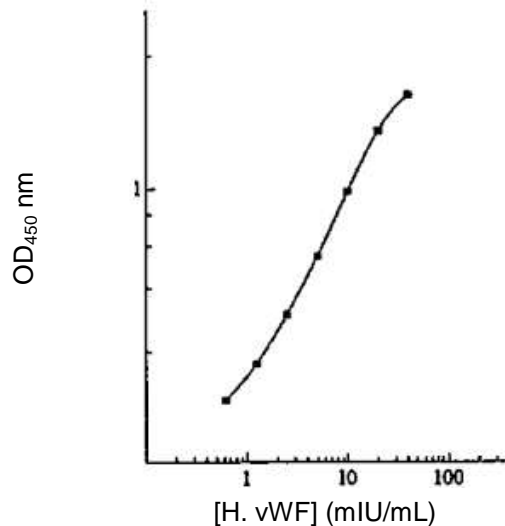
- ✓ Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- ✓ To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- ✓ Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.
- ✓ Typical Data

The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	mIU/mL	OD	Average OD
P1	40	2.078 2.014	2.046
P2	20	1.645 1.603	1.624
P3	10	1.172 1.144	1.158
P4	5.0	0.789 0.769	0.779
P5	2.5	0.509 0.495	0.502
P6	1.25	0.318 0.310	0.314
P7	0.625	0.235 0.229	0.232
P8	0.0	0.122 0.120	0.121
Sample: Pooled Normal, Sodium Citrate Plasma (100x)		1.052 1.034	1.043

- ✓ Standard Curve

The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



- ✓ Reference Value
- Normal human plasma VWF concentration has been reported ranging approximately from 0.3-1.57 IU/mL (4). For O blood group subjects, normal citrated human plasma VWF values are 0.52-1.54 IU/mL and for non-O blood group subjects, the values are 0.6-2.0 IU/mL (5).
- Plasma and serum samples from healthy adults were tested (n=40). On average, human VWF level was 1.0 IU/mL.

Sample	n	Average Value (IU/mL)
Pooled Normal Plasma	10	0.95
Normal Plasma	20	0.91
Pooled Normal Serum	10	1.15

Note 1: The conversion of IU and  $\mu\text{g}$  is  $1 \text{ IU/mL} = 9.8 \mu\text{g/mL}$ .

Note 2: The conversion of IU and mIU is  $1 \text{ IU/mL} = 1000 \text{ mIU/mL}$ .

### **Performance Characteristics**

- ✓ The minimum detectable dose of human VWF as calculated by 2SD from the mean of a zero standard was established to be 0.33 mIU/mL.
- ✓ Intra-assay precision was determined by testing three plasma samples twenty times in one assay.
- ✓ Inter-assay precision was determined by testing three plasma samples in twenty assays.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	5.5%	5.8%	4.9%	9.9%	10.5%	9.6%
Average CV (%)	5.4%			10.0%		

✓ Recovery

Standard Added Value	2.5 – 20 mIU/mL
Recovery %	91-112%
Average Recovery %	98%

✓ Linearity

Plasma and serum samples were serially diluted to test for linearity.

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
50x	93%	95%
100x	101%	102%
200x	107%	104%

✓ Cross-Reactivity

Species	Cross Reactivity (%)
Canine	None
Bovine	None
Monkey	< 40%
Mouse	None
Rat	< 15%
Swine	None
Rabbit	None

## Resources

### Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of improper components	Check the expiration date listed before use. Do not interchange components from different lots.
	Improper wash step	Check that the correct wash buffer is being used. Check that all wells are empty after aspiration. Check that the microplate washer is dispensing properly. If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.
	Improperly sealed microplate	Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing.
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	Each step of the procedure should be performed uninterrupted.
	Omission of step	Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	Check pipette calibration. Check pipette for proper performance.
	Wash step was skipped	Consult the provided procedure for all wash steps.
	Improper wash buffer	Check that the correct wash buffer is being used.
	Improper reagent preparation	Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	Consult the provided procedure for correct incubation time.

Deficient Standard Curve Fit	Non-optimal sample dilution	<p>Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay.</p> <p>Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay.</p> <p>User should determine the optimal dilution factor for samples.</p>
	Contamination of reagents	A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	<p>Pipette properly in a controlled and careful manner.</p> <p>Check pipette calibration.</p> <p>Check pipette for proper performance.</p>
	Insufficient mixing of reagent dilutions	<p>Thoroughly agitate the lyophilized components after reconstitution.</p> <p>Thoroughly mix dilutions.</p>

## **References**

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2. Okumura T. et al. (1976) Thromb. Res., 8:701
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**Plate Layout**

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3								
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	A	B	C	D	E	F	G	H