Human VWF
ELISA Kit

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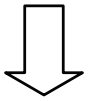
Thank You for choosing Assaypro

Hinweis/Note:
Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

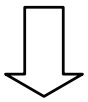
The datasheet is only a first information.
The relevant datasheet is included with the product.

Assay Summary

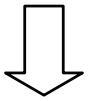
Add 50 µl of standard/sample per well.
Incubate 2 hours.



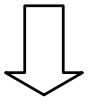
Wash, then add 50 µl of
biotinylated antibody per well.
Incubate 2 hours.



Wash, then add 50 µl of SP per well.
Incubate 30 minutes.



Wash, then add 50 µl of
Chromogen Substrate per well.
Incubate 20 minutes.



Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Assay Template

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								

AssayMax Human Von Willebrand Factor (VWF) ELISA Kit

Catalog No. EV2030-1
Sample Insert/Reference Only

Introduction

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

Caution and Warning

- **Prepare all reagents (working diluent buffer, wash buffer, standards, 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 protocol. 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.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.

- The Stop Solution is an acidic solution.

Reagents

- **Human VWF Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against VWF.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human VWF Standard:** Human VWF in a buffered protein base (80 mU, lyophilized, calibrated against WHO 1st International Standard).
- **Biotinylated Human VWF Antibody (80x):** A 80-fold concentrated biotinylated polyclonal antibody against VWF (100 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, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Store components of the kit at 2-8°C or -20°C upon arrival 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.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store standard at 2-8°C before reconstituting with diluent and at -20°C after reconstituting with diluent.

Other Supplies Required

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

Sample Collection, Preparation and Storage

- **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. Dilute samples 1:100 into MIX Diluent and assay. 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. Dilute samples 1:100 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. The samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles.

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 MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 80 mU of Human VWF Standard with 1 ml of MIX Diluent to generate a solution of 80 mU/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the VWF standard solution (80 mU/ml) 1:2 with equal volume of MIX Diluent to produce 40, 20, 10, 5, and 2.5 mU/ml solutions. MIX Diluent serves as the zero standard (0 mU/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[VWF] (mU/ml)
P1	Standard (80 mU/ml)	80.00
P2	1 part P1 + 1 part MIX Diluent	40.00
P3	1 part P2 + 1 part MIX Diluent	20.00
P4	1 part P3 + 1 part MIX Diluent	10.00
P5	1 part P4 + 1 part MIX Diluent	5.000
P6	1 part P5 + 1 part MIX Diluent	2.500
P7	MIX Diluent	0.000

- **Biotinylated Human VWF Antibody (80x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:80 with MIX Diluent. Any remaining solution should be frozen 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 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Human VWF Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 µl of Biotinylated Human VWF Antibody to each well and incubate for 2 hours.
- Wash the microplate as described above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for approximately 20 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- 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

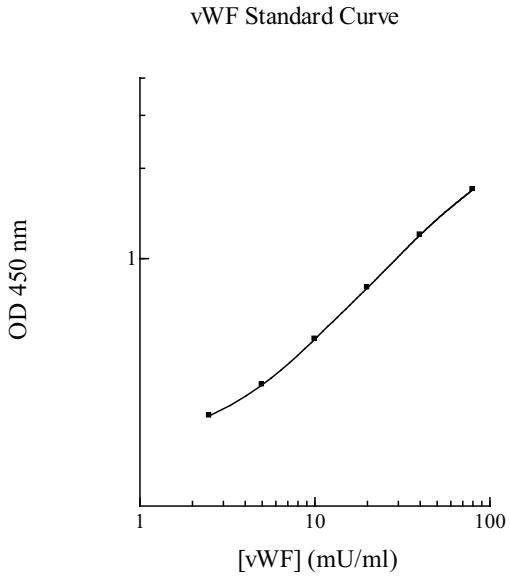
unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- 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 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.

Standard Curve

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



Performance Characteristics

- The minimum detectable level of VWF was typically ~ 2.5 mU/ml.
- Intra-assay and inter-assay coefficients of variation were 5.0% and 7.1% respectively.
- Standard has been calibrated against WHO reference standard.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:50	92%	93%
1:100	99%	98%
1:200	106%	103%

Recovery

Standard Added Value	3.0 - 30 mU/ml
Recovery %	87 - 118%
Average Recovery %	99%

Cross-Reactivity

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

Reference Values

- Normal human plasma VWF concentration has been reported ranging approximately from 0.3 to 1.57 IU/ml (4). Normal citrated human plasma VWF values are 0.52 – 1.54 IU/ml for O blood group subjects and 0.6 – 2.0 IU/ml for non-O blood group subjects (5).

Note

- The conversion of IU and µg is 1 International Unit (1 IU/ml) = 9.8 µg/ml.

References

(1) Zimmerman T.S. *et al.* (1987) *Human Pathology* 18:140
(2) Okumura T. *et al.* (1976) *Thromb. Res*., 8:701
(3) Morton L.F. *et al.* (1983) *Thromb. Res*., 32:545
(4) Inward CD *et al.* (1995) *Pediatr Nephrol*, 9(5): 574-8
(5) Pittet JL *et al.* (1997) Blood Coagul. Fibrinolysis 8:209-15

Version 7.5