



AssayMax Human Von Willebrand Factor (VWF) ELISA Kit

Catalog No. EV2030-1

Lot No.

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).

Principal of the Assay

The AssayMax VWF ELISA 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 murine antibody specific for VWF has been pre-coated onto a microplate. Human VWF in standards and samples is sandwiched by the immobilized monoclonal antibody and biotinylated polyclonal 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 acid solution

Reagents

- **VWF Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a murine monoclonal antibody against VWF.

- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **VWF Standard:** Human VWF in a buffered protein base, 2 vials (40 mU, lyophilized).
- **Biotinylated 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 concentrated (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 2-8⁰C or -20⁰C upon arrival up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20⁰C
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8⁰C
- Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.
- Diluent (1x) may be stored for up to 1 month at 2-8⁰C.
- Store Standard at 2-8⁰C before reconstituting with Diluent and at -20⁰C 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).
- 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 and assay. Dilute 10 µl of samples 1:100 with 990 µl of MIX Diluent. The undiluted samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant)
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes. Remove serum and assay. Dilute 10 µl of samples 1:100 with 990 µl of MIX Diluent. The undiluted samples can be stored at -20⁰C 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 -20⁰C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

- **MIX Diluent Concentrate (10x):** Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8^oC.
- **VWF Standard:** Reconstitute the 40 mU of human VWF Standard with 0.5 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 Standard solution (80 mU/ml) twofold with equal volume of MIX Diluent to produce 40, 20, 10, 5 and 2.5 mU/ml. MIX Diluent serves as the zero standard (0 mU/ml).

Standard Point	Dilution	[VWF] (mU/ml)
P1	1 part VWF 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 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^oC.
- **Wash Buffer Concentrate (20x):** Dilute Wash Buffer Conc. 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^oC.

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 (20-30^oC).
- 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.
- Add 50 µl of standard or sample per well, and cover wells and incubate for two 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 it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
- Add 50 µl of Biotinylated VWF Antibody to each well and incubate for two hours.
- Wash a microplate as described above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash a microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 15 minutes or until the optimal blue color density develops. Gently tap 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.

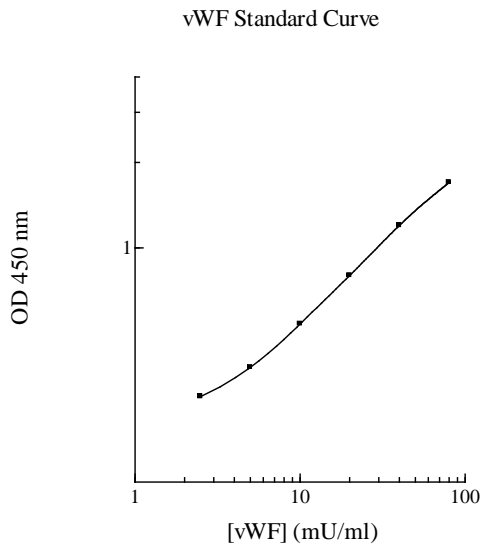
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may 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 mU/ml.
- Intra-assay and inter-assay coefficients of variation were 5.1% and 7.2% respectively.

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
1:50	96%	97%
1:100	101%	103%
1:200	106%	100%

Recovery

Standard Added Value	3 – 30 mU/ml
Recovery %	89-118 %
Average Recovery %	100.5 %

Cross-Reactivity

Species	% Cross Reactivity
Beagle	None
Bovine	None
Monkey	< 40 (suggest dilution 1:40 for plasma/serum)
Mouse	None
Rat	< 20 (Suggest dilution 1:10 for plasma/serum)
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).

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

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