AssayMax Human Von Wilebrand Factor (vWF) ELISA Kit

Catalog # EV2030-1
Lot #

Introduction

Von Wilebrand 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 server 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 3.5 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

- 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 (40 mU, lyophilized).
- **Biotinylated vWF Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against vWF (80 µl).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate)**: A 100-fold concentrate (120 µl).

- **EIA Diluent Concentrate (10x):** A 10-fold buffered protein base (30 ml).
- **Wash Buffer Concentrate (10x)**: A 10-fold concentrated buffered surfactant (2 x 30 ml).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydroxychloric acid (12 ml) to stop the chromogen substrate reaction.

Storage Condition

- Store unopened kit at 2-8^{0}C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8^{0}C. Store reconstituted standard at -20^{0}C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 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 3,000 x g for 10 minutes and assay. Dilute 10 µl of samples 1:100 with 990 µl of EIA Diluent. The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3,000 x g for 10 minutes. Remove serum and assay. Dilute 10 µl of samples 1:100 with 990 µl of EIA Diluent. The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3,000 x g for 10 minutes to remove debris. Collect supernatants and assay. The samples can be stored at -20^{0}C 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.
- **vWF Standard:** Reconstitute the 40 mU of human vWF Standard with 1 ml of EIA Diluent to generate a solution of 40 mU/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the Standard solution (40 mU/ml) twofold with equal volume of EIA Diluent to produce 20, 10, 5, 2.5, and 1.25 mU/ml. EIA Diluent serves as the zero standard (0 mU/ml).

Standard	Dilution	[vWF] (mU/ml)

Point		
P1	1 part vWF Standard (40 mU/ml)	40.00
P2	1 part P1 + 1 part EIA Diluent	20.00
P3	1 part P2 + 1 part EIA Diluent	10.00
P4	1 part P3 + 1 part EIA Diluent	5.00
P5	1 part P4 + 1 part EIA Diluent	2.50
P6	1 part P5 + 1 part EIA Diluent	1.25
P7	EIA Diluent	0.00

- **EIA Diluent Concentrate (10x):** Dilute EIA Diluent Conc. 1:10 with reagent grade water.
- **Biotinylated vWF Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with EIA Diluent.
- **Wash Buffer Concentrate (10x):** Dilute Wash Buffer Conc. 1:10 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent.

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^{0}C).
- 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 2 hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer. Invert the plate and decant the contents, and hit the plate 4-5 times on absorbent paper towel to complete remove liquid at each step.
- Add 50 µl of Biotinylated vWF Antibody to each well and incubate for 30 minutes.
- Wash five times with 200 µl of Wash Buffer as 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 five times with 200 µl of Wash Buffer as above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 10 minutes or till 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**. 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.

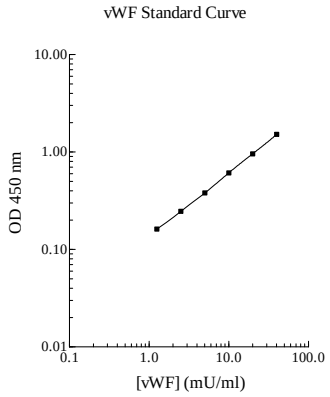
Data Analysis

- Calculate the mean value of the 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 of the linear portion using log-log curve fit.

- Determine the unknown sample concentration from the Standard Curve and multiply the plasma value by the dilution factor of 100.

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 less than 1 mU/ml.
- Intra-assay and inter-assay coefficients of variation were 4.9% and 7.6% respectively.
- No significant cross-reactivity or interference was observed.

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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