von Willebrand Factor (vWF) ELISA

Catalog Number: VWF31-K01
1 x 96 Wells
For Research Use Only (RUO). Not for use in clinical, diagnostic or therapeutic procedures.

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**Intended Use:**

The Eagle Biosciences von Willebrand Factor (vWF, factor VIII-related antigen) ELISA assay kit is intended for the quantitative determination of von Willebrand Factor (vWF) in plasma by enzyme linked immunoassay (ELISA). The von Willebrand Factor (vWF) ELISA assay kit is for research use only and not to be used in diagnostic procedures.

**Assay Background:**

The Eagle Biosciences von Willebrand Factor (vWF, factor VIII-related antigen) ELISA assay kit is an enzyme-linked immunoassay (ELISA) for quantifying von Willebrand factor in human plasma. Studies have shown that this plasma protein factor is deficient or defective in patients with von Willebrand's disease, a relatively common inherited disorder associated with an increased tendency to bleed. Laboratory studies in these patients usually demonstrate:

1) a decrease in procoagulant activity as measured by activated partial thromboplastin time
2) a prolonged bleeding time
3) decreased plasma concentration of vWF.

It can useful to differentiate samples with von Willebrand disease from those with Classical Hemophilia A (Factor VIII coagulant deficiency). The latter group has prolonged activated partial thromboplastin time (PTT) but have normal or increased concentrations of von Willebrand Factor (vWF) in their plasma. In contrast, samples with von Willebrand disease have depressed levels of vWF.

**Principle of Procedure:**

Eagle’s enzyme immunoassay for the non-radioactive quantitative determination of human von Willebrand Factor is a 2 step assay:

Step 1: Diluted plasma specimens, Calibrators, and Sample Diluent are pipetted into individual microtiter wells precoated with rabbit anti-vWF antibody. Horseradish peroxidase (HRP) conjugated antibody (sheep) is added to the wells and the plate is incubated. During incubation, vWF in each specimen and Calibrator diffuse throughout the well and are captured on the well surface. Concurrently, the HRP-conjugated antibody reacts with other sites on vWF antigens in the specimen and Calibrators forming a sandwich immobilized on the wall of the microtiter well.
Step 2: The microtiter wells are washed four times to remove the non-bound reactants. The remaining HRP-labeled complex is detected by the addition of TMB, a substrate specific for the HRP enzyme. After adding the TMB, a blue color develops in proportion to the amount of vWF in each well. After a short incubation, a mild acid is added to stop the substrate conversion and change the color from blue to yellow. The yellow color is measured in a microplate reader using a 450nm filter. The amount of vWF present in each specimen is then determined by interpolating from a graph of the adsorption vs. concentration for the Calibrators.

The intensity of the color developed during the 2nd Step is directly proportional to both time and temperature; therefore an increase in the 2nd Step incubation time will increase the intensity of the color, while a decrease in the 2nd Step incubation time will decrease the intensity of the color. The same applies for an increase or a decrease in the incubation temperature. It is recommended that time rather than temperature be used to optimize absorbance.

Materials Provided:

- All reagents in this kit should be stored at 2 - 8°C. Refer to the expiration date on the label of each reagent. Upon reconstitution, the Calibrators are stable for a maximum of 4 weeks, when stored a 2 - 8°C.

**Human von Willebrand Factor Calibrator**: 2 vials containing 0.5ml of prediluted lyophilized human plasma with a known vWF concentration, borate saline buffer, bovine serum albumin, sheep serum, EDTA and ProClin 300TM as a preservative. The concentration of vWF in the Calibrators was determined by comparison to World Health Organization International Reference material.

At least fifteen minutes prior to use, reconstitute a vial of the Calibrator by carefully adding exactly 0.50ml of deionized, distilled water to the vial, briefly vortexing to mix and solubilize the diluted plasma, and allow to stand at room temperature for 15 minutes prior to use. The reconstituted Calibrator solution is stable at 2 - 8°C for a maximum of 4 weeks.

**Microtiter Wells**: 8 (eight) 12 x 1 microtiter well Strips (96 total wells) coated with rabbit immunoglobulin isolated from antiserum specific for human vWF and stabilized in dry form. The Wells are provided in a resealable foil pouch containing a desiccant packet.

**Plate Sealing Tape**: 2 Sheets

**Microplate Frame**: 1
**Specimen Diluting Buffer:** 1 vial containing 45 ml of borate-saline buffer, bovine serum albumin, normal rabbit serum, normal sheep serum, ethylenediaminetetraacetate (EDTA) and ProClin 300TM as a preservative.

**HRP-Conjugated Anti-vWF:** 1 vial containing 22 ml of HRP-conjugate sheep antibody in a diluent of saline, normal sheep serum, a commercial stabilizing agent and ProClin 300TM as a preservative.

**Substrate:** 1 amber plastic bottle containing 22 ml of 3,3',5,5'-tetramethylbenzidine (TMB) one component substrate, in an acidic buffer, with 0.01% hydrogen peroxide. Because of its extreme sensitivity, avoid exposing this reagent to light, borosilicate glass and elevated temperatures. As supplied, the Substrate should be colorless, slightly amber or slightly bluish tinted. **DO NOT USE THIS SUBSTRATE IF IT IS DEEPLY BLUE IN COLOR BEFORE ADDITION TO THE MICROWELLS.**

At the final stage of incubation, remove from refrigerator and measure the amount of Substrate required for the current assay, using only plastic pipet materials, recap the bottle and return to 2 - 8°C storage. The material removed should be stored in the dark and allowed to slowly come to ambient temperature prior to addition to the washed microwells.

**Stop Solution:** 1 vial containing 12 ml of 1% hydrochloric acid. **Caution:** Caustic material. Wear eye, hand, face and clothing protection.

**5X Wash Solution:** 1 vial containing 50ml of concentrated buffer solution with surfactant.

**Materials required but not provided**

- Distilled, deionized water
- 20 µl, 50 µl, 200 µl, and 500 µl precision pipettes
- Microplate Reader capable of reading at 450nm (Optional: a background correction filter set at 570 to 630 nm)
- 12 x 75 mm plastic culture tubes - DO NOT USE GLASS TUBES
- Vortex mixer
- Wash bottle
- Graduated cylinder capable of measuring 250 ml of liquid Microplate washing device or wash bottle.
- Laboratory horizontal rotator table (Optional).
- Statistical/Scientific calculator with regression program or a computer program for point to point or polynomial curve fitting (Optional).
Precautions:

- The human von Willebrand Factor (vWF) ELISA assay kit is for research use only and not to be used in clinical, therapeutic or diagnostic procedures.
- The Acid Stop Solution is a solution of hydrochloric acid. Wear appropriate eye, hand, face and clothing protection when using this material.
- Standards provided in the von Willebrand Factor (vWF) ELISA assay kit contain purified protein obtained from human donors. Each donor’s serum was tested by FDA approved methods for the presence of antibodies to HIV-1 as well as for hepatitis B surface antigen and found to be negative. However, since no test method can absolutely guarantee the absence of these or other infectious agents, all standards, controls and human sera tested with this assay should be handled in accordance with NCCLS guidelines for preventing the transmission of blood-borne infections during laboratory procedures. Wear gloves and avoid contact with skin and mucous membranes.
- von Willebrand Factor (vWF) ELISA assay kit reagents should not be used beyond the expiration date.
- Avoid exposure of reagents to strong light during incubation and storage.
- Perform the von Willebrand Factor (vWF) ELISA assay kit only as provided and described in this product insert.
- Substitution of kit reagents and/or alteration of incubation times or conditions may lead to erroneous results.
- Use only high quality (deionized and filtered or distilled) water for dilution of the 5X Wash Solution.

Sample Preparation:

1. Remove all reagents, except the Substrate, from cold storage and allow to warm to room temperature.

2. Use freshly collected plasma specimens anti-coagulated with citrate or EDTA.

3. Centrifuge to separate the plasma from the red cells (if the assay is to be performed later, freeze the plasma and store at -20°C or lower). Thaw frozen plasma samples by rapidly moving the storage vial/tube in a 37°C water bath until thawed (approximately 30 - 60 seconds for a 50 µl sample) followed immediately by a brief vortex.

4. Carefully remove the aluminum seal and rubber stopper from the Calibrator vial. Be careful not to remove or lose any powder from the vial. If any material is lost prior to reconstitution, discard and use another vial of Calibrator. Add exactly 0.50 ml of distilled, deionized water to the vial. Replace the rubber stopper and mix well, including vortexing for 5 - 10 seconds. Allow the reconstituted Calibrator to stand for at least 15 minutes.
5. Dilute each patient sample 1:51 prior to testing. Add 1 ml of Specimen Diluting Buffer to a labeled 12 x 75 mm plastic tube for each specimen to be tested. Add 20 µl of specimen plasma sample to 1 ml of Specimen Diluting Buffer in plastic test tubes. DO NOT USE GLASS TUBES.

6. Label 5 separate plastic tubes as 1:2, 1:4, 1:8, 1:16 and 1:32. To each tube, add exactly 200 µl of Specimen Diluting Buffer.

7. Remove 200 µl of reconstituted pre-diluted Calibrator from the vial and carefully add to the specimen diluent in the tube labeled "1:2". Cap the tube and vortex for several seconds to mix thoroughly. Using a fresh pipet tip, remove 200 µl from this tube ("1:2") and add to the Specimen Diluting Buffer in the tube labeled "1:4". Cap the tube and vortex for several seconds to mix thoroughly. Using a fresh pipet tip, remove 200 µl from this tube ("1:4") and add to the tube labeled "1:8". Cap the tube and vortex to mix thoroughly. Repeat this process for the tube marked "1:16" and "1:32".

These five dilutions, as well as the reconstituted Calibrator, should be tested in duplicate each time the assay is performed so that an accurate calibration curve can be constructed for the determination of vWF concentrations in the specimens. It is recommended that each individual laboratory locate and use controls for this assay that are representative of the population upon which this test is performed. Routine use of these controls will allow each laboratory to perform ongoing quality control to identify any performance or reagent problems that may occur.

**Reagent Preparation:**

Wash Solution: Prepare a 1X Wash Solution by pouring the entire contents of the 5X Wash Solution into a clean 250 ml graduated cylinder. Bring the total volume to 250 ml with distilled water. Cover and invert to mix. Transfer to a clean wash bottle and store at room temperature.

**Assay Procedure:**

Bring all reagents to room temperature before beginning von Willebrand Factor (vWF) ELISA assay kit.

1. Determine the number of Wells/Strips that will be required for the assay run. (Note: each specimen, Calibrator dilution, and Specimen Buffer should be run in duplicate wells each time the assay is performed.)
2. Open the foil pouch and remove the number of 12-well Strips required for each assay. Place the Strips in the frame supplied, being careful to orient the strips correctly. Replace the unused Strips in the resealable foil pouch. Reseal the pouch, using the ziploc strip, and store at 2 - 8°C.

3. Pipette 20 µl Specimen Diluting Buffer to each of Wells A1 and B1. With a new pipet tip, remove and add 20 µl of Calibrator from its vial to each of Wells A2 and B2. With a new pipet tip, remove and add 20 µl of Calibrator from the tube marked "1:2" and add to each of Wells A3 and B3. In the same manner, add 20 µl of Calibrators from the tubes marked 1:4, 1:8, 1:16 and 1:32.

4. Add 20 µl of the 1:51 dilutions of the patient specimens and controls, in duplicate, to the remaining Wells.

5. Pipette 200 µl of HRP-Conjugate to each well containing Calibrators, patient specimens and Specimen Diluting Buffer (0% or NSB). Cover the Strips immediately with a Plate Sealer, being careful to press the Sealer onto all Wells.

6. Place the plate on a mixing table and mix for 10 minutes at 190 - 200 rpm. Remove the plate and store undisturbed on a flat surface at room temperature (16 - 25°C) for two hours.

7. Prepare a 1X Wash Solution by pouring the entire contents of the 5X Wash Solution into a clean 250 ml graduated cylinder. Bring the total volume to 250 ml with distilled water. Cover and invert to mix. Transfer to a clean wash bottle and store at room temperature.

8. After the two hour incubation, remove the Plate Sealer and dump the contents into a sink or waste receptacle. Blot the plate to remove reactants from the Well lips. Wash the Wells by filling each Well with the previously prepared 1X Wash Solution from the wash bottle. Dump the contents and repeat this wash
procedure three additional times (total of four washes). After the final wash, dump the wash fluid and then vigorously tap the Strips on an absorbent surface to remove all liquids from the Wells.

Make sure there are no bubbles or liquid remaining in the Wells. If any fluid remains, continue tapping the plate on the absorbent surface until eliminated. Do not add Substrate to the Wells until the Wells have been tapped dry and are free of bubbles.

9. Using new pipet tips, add 200 µl of Substrate to each Well. Place the Wells into a dark area, such as a drawer, and incubate undisturbed for 30 minutes.

10. Carefully remove the plate from the dark and add 50 µl of the Stop Solution to each Well. Place the plate on the mixing table for 30 seconds at 190 rpm to ensure mixing. Read the absorbance of the Wells at 450nm. If possible, use a correction wavelength of 570 - 630nm.

11. If automatic background subtraction is not available, it is suggested that the plate be read a second time at 570 - 630nm and these readings be manually subtracted from the initial 450nm readings. Failure to compensate for the background absorbance may increase the variability of the assay and result in potentially erroneous values.

**Calculations:**

Average the duplicate readings for each specimen, Calibrator, and Specimen Diluting Buffer. Subtract the average absorbance of the Specimen Diluting Buffer from all other averages to obtain the corrected mean absorbances.

Manual Data Analysis: On the graph paper provided, plot the corrected net absorbance values for the prediluted Calibrator and its two-fold dilutions on the Y axis and plot the von Willebrand Factor concentration supplied in the kit for the prediluted Calibrator on the X axis. The vWF concentrations of the two-fold dilutions will be 1/2, 1/4, 1/8 etc. of the value reported on the label.
Draw the straightest line possible that intersects the points plotted on the graph paper. This is the assay calibration line. To determine the vWF concentration of each patient specimen, locate the mean corrected net absorbance of the specimen on the Y axis and then locate the corresponding point on the calibration line determined previously. Slide a ruler down paralleling the Y axis and determine the corresponding value of the intersect point on the X axis. This value on the X axis is the concentration of the vWF present in the undiluted patient specimen, expressed as a percentage of normal concentration (%N).

Automated Data Analysis: Best fit for a calibrator line plot can be most often described by a third order polynomial plot of the calibrator (and dilutions) absorbances versus the vWF concentrations. Using the polynomial equation for the line which results, determine the vWF concentrations in the unknown specimens by interpolation of their corrected mean absorbances. The results will be expressed as a percentage of normal values (%N).

Do not extrapolate values that are outside the highest values determined for the calibration line. If values are obtained that are greater than the highest vWF value plotted, they can either be reported as values greater than the value of the highest Calibrator, or diluted further and retested.

The 1:51 dilution may be diluted 1:2 in Specimen Diluting Buffer and retested. The 1:51 dilution may be further diluted and tested immediately or stoppered and frozen at -20C (or less) for no more than 30 days after the original dilution was assayed. If the specimens are diluted and retested, the values reported must be corrected after assay by a factor of 2 to correct for the dilution.

**Typical Data** (for Demonstration Purposes Only)

<table>
<thead>
<tr>
<th>STANDARD</th>
<th>O.D.</th>
<th>MEAN</th>
<th>Net O.D.*</th>
<th>VWF CONC. % of NORMAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% or NSB</td>
<td>0.032 / 0.036</td>
<td>0.034</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Calibrator</td>
<td>1.477 / 1.472</td>
<td>1.475</td>
<td>1.411</td>
<td>200%</td>
</tr>
<tr>
<td>1:2 Calibrator</td>
<td>1.038 / 1.043</td>
<td>1.041</td>
<td>1.007</td>
<td>100%</td>
</tr>
<tr>
<td>1:4 Calibrator</td>
<td>0.624 / 0.653</td>
<td>0.639</td>
<td>0.605</td>
<td>50%</td>
</tr>
<tr>
<td>1:8 Calibrator</td>
<td>0.341 / 0.368</td>
<td>0.355</td>
<td>0.321</td>
<td>25%</td>
</tr>
<tr>
<td>1:16 Calibrator</td>
<td>0.192 / 0.192</td>
<td>0.192</td>
<td>0.158</td>
<td>12.50%</td>
</tr>
<tr>
<td>1:32 Calibrator</td>
<td>0.107 / 0.112</td>
<td>0.11</td>
<td>0.076</td>
<td>6.25%</td>
</tr>
</tbody>
</table>
**Expected Values**

Samples with the relatively common classical (Type I) form of von Willebrand's disease have plasma vWF levels that are usually in the 5 - 40% of normal range. Samples with relatively rare severe forms of von Willebrand's disease have plasma levels that are often less than 5% of normal. Some samples with von Willebrand disease have faulty molecules which do not polymerize properly (Type II). These samples may have normal von Willebrand antigen concentrations.

Individuals with classical Hemophilia A (deficiency of factor VIII coagulant activity) generally have vWF antigen levels within or above the normal range. Female carriers of Hemophilia A usually have a ratio of plasma factor VIII coagulant activity/vWF antigen that is approximately 1:2.

**Reproducibility:**

Within and between assay variations were calculated using repeated analysis of a panel of reference plasma samples. Within assay variance was determined by assaying and individually comparing 10 repetitions of 8 samples. Between assay variance was determined by individual comparison of 35 samples each run on 9 separate occasions.

**Intra-Assay**

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Mean vWF</th>
<th>Standard Deviation</th>
<th>% C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>165</td>
<td>8.2</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>145</td>
<td>3.2</td>
<td>4.3</td>
</tr>
<tr>
<td>3</td>
<td>63</td>
<td>3.7</td>
<td>5.8</td>
</tr>
<tr>
<td>4</td>
<td>62</td>
<td>30</td>
<td>4.8</td>
</tr>
<tr>
<td>5</td>
<td>59</td>
<td>2.6</td>
<td>4.4</td>
</tr>
<tr>
<td>6</td>
<td>37</td>
<td>1.8</td>
<td>4.8</td>
</tr>
<tr>
<td>7</td>
<td>37</td>
<td>1.9</td>
<td>5.3</td>
</tr>
<tr>
<td>8</td>
<td>19</td>
<td>1.4</td>
<td>7.2</td>
</tr>
</tbody>
</table>
Inter-Assay:

<table>
<thead>
<tr>
<th>TEST GROUPS</th>
<th>CONC. VWF</th>
<th>No. of Samples</th>
<th>vWF Ag Mean</th>
<th>S.D.</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 - 10 %</td>
<td>1</td>
<td>8</td>
<td>0.6</td>
<td>7.5</td>
</tr>
<tr>
<td>2</td>
<td>11 - 49 %</td>
<td>11</td>
<td>35</td>
<td>2.8</td>
<td>7.8</td>
</tr>
<tr>
<td>3</td>
<td>50 - 160 %</td>
<td>16</td>
<td>84</td>
<td>5.9</td>
<td>7.0</td>
</tr>
<tr>
<td>4</td>
<td>161 - 200+ %</td>
<td>7</td>
<td>211</td>
<td>24.5</td>
<td>11.5</td>
</tr>
</tbody>
</table>

Sensitivity:

Sensitivity is defined as the smallest amount of vWF which can be distinguished from zero with a 95% confidence limit (+ or - two standard deviations). The smallest concentration of vWF that can be distinguished from zero with this von Willebrand Factor (vWF) ELISA assay kit is 0.78% of Normal.

Accuracy:

The accuracy of the Willebrand Factor (vWF) ELISA assay was determined by comparing the quantity of von Willebrand Factor determined by this test procedure with the quantity of von Willebrand factor determined by an approved bead ELISA test.

One hundred and eighteen (118) plasma samples were obtained from four groups of patients and normal volunteers. These samples were assayed for the quantity of vWF by the bead ELISA and by this procedure. The correlation coefficient of the bead ELISA and this test procedure was \( r = 0.849 \) by linear regression analysis.

Bibliography:


**Warranty Information**

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