Instructions for Use

For the quantitative measurement of Human Von Willebrand Factor (VWF) in cell culture supernatants, plasma and serum

This product is for research use only and is not intended for diagnostic use.
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1. Introduction

Von Willebrand factor (VWF) is a multimeric glycoprotein that circulates in blood forming a noncovalent complex with procoagulant factor VIII. 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. The congenital dysfunctional state of VWF causes a moderate to severe bleeding diathesis-von Willebrand disease (VWD).

ab108918 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.
2. Assay Summary

Prepare all reagents, samples and standards as instructed.

Add 50 µl standard or sample to each well.
Incubate 2 hours at room temperature.
Wash the microplate 5 x with wash buffer.
Add 50 µl prepared biotin antibody to each well.
Incubate 2 hours at room temperature.
Wash the microplate 5 x with wash buffer.
Add 50 µl of Streptavidin-Peroxidase Conjugate.
Incubate 30 minutes at room temperature.
Wash the microplate 5 x with wash buffer.
Add 50 µl of Chromogen Substrate to each well.
Incubate 15 minutes or till the optimal blue colour density develops.
Add 50 µl Stop Solution to each well. Read at 450 nm immediately.
3. Kit Contents

- 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, 1 vial (lyophilized).
- Biotinylated VWF Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against VWF (140 µl).
- 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).
4. Storage and Handling

- 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
- 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 2-8°C.
- Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

5. Additional Materials Required

- Microplate reader capable of measuring absorbance at 450nm.
- Precision pipettes to deliver 1 µl to 1 ml volumes.
- Distilled or deionized reagent grade water.
6. Preparation of Reagents

Sample Collection:

1. **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 Diluent. 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 anticoagulant)

2. **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 Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

3. **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:

1. 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.

2. **Diluent (10x):** Dilute the Diluent 1:10 with reagent grade water. Store for up to 1 month at 2 to 8 °C.

3. **Standard Curve:** VWF Standard: Reconstitute the Human VWF Standard with an appropriate volume of 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 Diluent to produce 40, 20, 10, 5 and 2.5 mU/ml. Diluent serves as the zero standard (0 mU/ml).
<table>
<thead>
<tr>
<th>Standard Point</th>
<th>Dilution</th>
<th>[VWF] (mU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Standard (80mU/ml)</td>
<td>80.00</td>
</tr>
<tr>
<td>P2</td>
<td>1 part P1 + 1 part Diluent</td>
<td>40.00</td>
</tr>
<tr>
<td>P3</td>
<td>1 part P2 + 1 part Diluent</td>
<td>20.00</td>
</tr>
<tr>
<td>P4</td>
<td>1 part P3 + 1 part Diluent</td>
<td>10.00</td>
</tr>
<tr>
<td>P5</td>
<td>1 part P4 + 1 part Diluent</td>
<td>5.00</td>
</tr>
<tr>
<td>P6</td>
<td>1 part P5 + 1 part Diluent</td>
<td>2.50</td>
</tr>
<tr>
<td>P7</td>
<td>Diluent</td>
<td>0.00</td>
</tr>
</tbody>
</table>

4. **Biotinylated VWF Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with Diluent. Any remaining solution should be frozen at -20°C.

5. **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.

6. **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with Diluent. Any remaining solution should be frozen at -20°C.
7. Assay Method

1. 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°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 µl of standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.

4. Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; tap 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; tap it 4-5 times on absorbent paper towel to completely remove the liquid.

5. Add 50 µl of Biotinylated VWF Antibody to each well and incubate for two hours.

6. Wash microplate as described above.

7. 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.

8. Wash microplate as described above.
9. Add 50 µl of Chromogen Substrate per well and incubate for about 15 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.

10. Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.

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.

8. 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. Note: The conversion of IU and µg is 1 International Unit (1 IU/ml) = 9.8 µg/ml
A. Typical Data

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

B. Sensitivity

The minimum detectable dose of VWF is typically 2.5 mU/ml.

C. Recovery

Standard Added Value: 3 – 30 mU/ml

Recovery %: 89-118

Average Recovery %: 100.5
D. Reproducibility

Intra-Assay: CV = 5.1%

Inter-Assay: CV = 7.2%

E. Linearity

<table>
<thead>
<tr>
<th>Sample Dilution</th>
<th>Average % of Expected Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
</tr>
<tr>
<td>1:50</td>
<td>97</td>
</tr>
<tr>
<td>1:100</td>
<td>100</td>
</tr>
<tr>
<td>1:200</td>
<td>105</td>
</tr>
</tbody>
</table>
9. Specificity

Normal human plasma VWF concentration has been reported ranging approximately from 0.3 to 1.57 IU/ml. 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.

<table>
<thead>
<tr>
<th>Species</th>
<th>% Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>None</td>
</tr>
<tr>
<td>Mouse</td>
<td>None</td>
</tr>
<tr>
<td>Rabbit</td>
<td>None</td>
</tr>
<tr>
<td>Rat</td>
<td>&lt;20 (Suggest 1:10 for Plasma/serum)</td>
</tr>
<tr>
<td>Swine</td>
<td>None</td>
</tr>
<tr>
<td>Monkey</td>
<td>&lt;40 (Suggest 1:40 for Plasma/serum)</td>
</tr>
<tr>
<td>Canine</td>
<td>None</td>
</tr>
</tbody>
</table>
## 10. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Improper standard dilution</td>
<td>Confirm dilutions made correctly</td>
</tr>
<tr>
<td></td>
<td>Standard improperly reconstituted (if applicable)</td>
<td>Briefly spin vial before opening; thoroughly resuspend powder (if applicable)</td>
</tr>
<tr>
<td></td>
<td>Standard degraded</td>
<td>Store sample as recommended</td>
</tr>
<tr>
<td></td>
<td>Curve doesn't fit scale</td>
<td>Try plotting using different scale</td>
</tr>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4 °C</td>
</tr>
<tr>
<td></td>
<td>Target present below detection limits of assay</td>
<td>Decrease dilution factor; concentrate samples</td>
</tr>
<tr>
<td></td>
<td>Precipitate can form in wells upon substrate addition when concentration of target is too high</td>
<td>Increase dilution factor of sample</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
<tr>
<td></td>
<td>Sample prepared incorrectly</td>
<td>Ensure proper sample preparation/dilution</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Large CV</th>
<th>Bubbles in wells</th>
<th>Ensure no bubbles present prior to reading plate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed wash wells as recommended</td>
</tr>
<tr>
<td></td>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
</tr>
<tr>
<td></td>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes and ensure accurate pipetting</td>
</tr>
<tr>
<td></td>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation and optimal sample storage conditions (eg. minimize freeze/thaws cycles)</td>
</tr>
</tbody>
</table>

| High background | Wells are insufficiently washed | Wash wells as per protocol recommendations |
|                 | Contaminated wash buffer | Make fresh wash buffer |
|                 | Waiting too long to read plate after adding STOP solution | Read plate immediately after adding STOP solution |

| Low sensitivity | Improper storage of ELISA kit | Store all reagents as recommended. Please note all reagents may not have identical storage requirements. |
|                 | Using incompatible sample type (e.g. Serum vs. cell extract) | Detection may be reduced or absent in untested sample types |

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select “contact us” on www.abcam.com for the your region).
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