

# ZYMUTEST™ vWF:CBA

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96 tests



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ELISA method for the quantitative determination of von Willebrand Factor  
Collagen Binding Activity.

**FOR RESEARCH USE ONLY.**

**DO NOT USE IN DIAGNOSTIC PROCEDURES.**

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## INTENDED USE:

The ZYMUTEST™ vWF:CBA kit is an ELISA method for the quantitative determination of human von Willebrand Factor (vWF) Collagen Binding Activity (CBA) on human plasma.

**This kit is for research use only and must not be used for patient diagnosis or treatment.**

## SUMMARY AND EXPLANATION:

### Technical:

vWF is a multimeric protein produced in endothelial cells and megakaryocytes. It circulates in blood as multimers ranging from 500 to more than 20,000 kDa.

vWF mediates platelet adhesion to subendothelium of the damaged blood vessel and, by complexing to Factor VIII, extends its half-life into the bloodstream.

Ultra-large multimers are proteolytically cleaved by ADAMTS13 into less active vWF forms. The biological function of vWF depends largely on the size of its multimers. Larger multimers are more likely to bind to platelets and collagen, and to promote platelet adhesion in circulating blood<sup>1,2</sup>.

## PRINCIPLE:

In a first step, the diluted tested plasma or specimen is introduced into a microwell coated with fibrillar collagen. When present, vWF is captured onto the solid phase through its collagen binding activity. Following a washing step, the immunoconjugate, which is a polyclonal antibody coupled to horse radish peroxidase (HRP), is introduced, and binds to free epitopes of immobilized vWF. Following a washing step, the peroxidase substrate, 3,3',5,5'-Tetramethylbenzidine (TMB), in presence of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), is introduced in the microwell plate and a blue colour develops. When the reaction is stopped with Sulfuric Acid, a yellow colour is obtained. The amount of colour developed is directly proportional to the concentration of human vWF:CBA in the plasma or in the tested sample.

## REAGENTS:

- COAT ELISA microplate** : 12x8 containing 12 strips of 8 wells, coated with collagen (equine, type I and III), stabilized and packed in an aluminium pouch in presence of a desiccant.
- SD ELISA Sample Diluent** : 2 vials of 50 mL, ready to use. Contains Proclin and BSA.
- CAL vWF vWF calibrator** : 3 vials of 2 mL. Lyophilized human plasma containing a titrated quantity of vWF in the range 120-160%. The standard concentration were established by reference to the NIBSC international standard. Contains BSA.
- CI vWF vWF Control high** : 1 vial of 0.5 mL, lyophilized.
- CII vWF vWF Control low** : 1 vial of 0.5 mL, lyophilized.
- IC ANTI-(h)-vWF HRP Anti-(h)-vWF-HRP immunoconjugate** : 3 vials of 7.5 mL, rabbit polyclonal antibody, specific of vWF and coupled to peroxidase, lyophilized. Contains BSA.
- CD ELISA Conjugate diluent** : 1 vial of 25 mL, ready to use. Contains Proclin and BSA.
- WS ELISA Wash solution** : 1 vial of 50 mL, 20x 20 fold concentrated. Contains Proclin.
- TMB 3,3',5,5'-Tetramethylbenzidine** : 1 vial of 25 mL of substrate, ready to use. Contains hydrogen peroxide.
- Stop 0.45M Sulfuric acid** : 1 vial of 6 mL, ready to use.

## WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of animal origin. Users of reagents of these types must exercise extreme care in full compliance with safety precautions in the manipulation of these biological materials as if they were infectious.
- Sulfuric acid, although diluted to 0.45M is caustic. As for any similar chemical, handle Sulfuric acid with great care. Wear protection glasses and gloves when handling. Avoid any skin and eye contact.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.
- Aging studies show that the reagents can be shipped at room temperature without degradation.
- This device of *in vitro* use is intended for professional use in the laboratory.

## REAGENT PREPARATION:

Allow the strips and reagents to stabilize for at least 30 min at room temperature before use. Gently remove the freeze-drying stopper, to avoid any product loss when opening the vial.

**COAT** Open the aluminum pouch and take off the required amounts of strips for the test series. The strips must be used within 30 minutes

Reconstitute the contents of each vial with exactly:

**CI vWF** → 0.5 mL of distilled water at least 30 min before use. Shake vigorously until complete dissolution.

**CII vWF** → 0.5 mL of distilled water at least 30 min before use. Shake vigorously until complete dissolution.

**CAL vWF** → 2 mL of **SD ELISA** in order to obtain a solution containing "C" % of vWF:CBA (already diluted at 1:50). Shake vigorously until complete dissolution.

**IC ANTI-(h)-vWF HRP** → 7,5 mL of **CD ELISA** at least 15 minutes before use. Shake gently until complete dissolution.

**SD ELISA** **TMB** **Stop** **CD ELISA**

Reagent ready to use.

**WS ELISA** Shake the vial and dilute the wash solution 1:20 in distilled water (the 50 mL of concentrated solution allow to prepare 1 liter of wash solution after dilution).

Incubate, if necessary, the vial in a water bath at 37°C, until complete dissolution of solids.

## STORAGE AND STABILITY:

Unopened reagents should be stored at 2-8°C in their original packaging. Under these conditions, they can be used until the expiry date printed on the kit.

**COAT** Unused strips can be stored at 2-8°C for 4 weeks in their original aluminum pouch (hermetically closed, in presence of the desiccant), stored in the provided plastic microplate storage bag (minigrip), protected from any moisture.

Reagent stability after reconstitution, free from any contamination or evaporation, and stored closed, is of:

**CAL vWF** → 8 hours at room temperature (18-25°C).

**CI vWF** **CII vWF**  
→ 24 hours at 2-8°C.  
8 hours at room temperature (18-25°C).  
2 months frozen at -20°C or less\*

**IC ANTI-(h)-vWF HRP**  
→ 4 weeks at 2-8°C.  
24 hours at room temperature (18-25°C).

\*Thaw only once, as rapidly as possible at 37°C and use immediately.

Reagent stability after opening, free from any contamination or evaporation, and stored closed, is of:

**SD ELISA** **CD ELISA** **TMB**  
→ 4 weeks at 2-8°C.  
**WS ELISA** → 4 weeks at 2-8°C.  
7 days at 2-8°C for the diluted solution.  
**Stop** → 8 weeks at 2-8°C.

## REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

### Reagents:

- Distilled water.

### Materials:

- 8-channel or repeating pipette allowing dispensing volumes of 50-300 µL.
- Pipettes at variable volumes from 0 to 20 µL, 20 to 200 µL and 200 to 1000 µL.
- Micro ELISA plate washing equipment and shaker.
- Micro ELISA plate reader with a wavelength set up at 450 nm.

## SPECIMEN COLLECTION AND PREPARATION:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture. Discard the first tube.

Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI GP44-A4<sup>5</sup> (and CLSI H21-A5<sup>6</sup>) guideline for further information concerning specimen collection, handling and storage).

For plasma storage, please refer to references.

## PROCEDURE:

### Assay method:

1. Specimens and controls should be diluted using **SD ELISA** as described in the table below:

Specimens	Dilution
CI vWF and CII vWF	1:50
Specimens	1:50

For expected vWF concentrations above "C"%, dilute specimens at **1:100** (D=100), or more.

2. Using the Calibrator **CAL vWF** with a concentration "C" in %, prepare the calibration range as described in the table below:

Concentration of vWF (%)	C	C:2	C:4	C:10	C:20	0
Vol. calibrator vWF	1 mL	0.5 mL	0.25 mL	0.1 mL	0.05 mL	0 mL
Vol. of Sample Diluent	0 mL	0.5 mL	0.75 mL	0.9 mL	0.95 mL	1 mL

Mix for homogenization.

The dilutions are stable for **6 hours** at room temperature (18-25°C).

3. Put strips in the frame provided. Introduce the reagents in the micro ELISA plate wells and perform the assay as indicated on the following table:

Reagent	Volume	Procedure
<b>CAL vWF</b> or <b>CI vWF</b> or <b>CII vWF</b> or Specimens to test diluted or <b>SD ELISA</b> (blank)	200µL	Introduce the standard solutions or the tested specimen in the corresponding micro ELISA plate well
<b>Incubate for 2 hours at room temperature (18-25°C) (a)</b>		
<b>WS ELISA</b>	300µL	Proceed to 10 successive washings (b)
<b>IC ANTI-(h)-vWF HRP</b>	200µL	Introduce the <b>IC ANTI-(h)-vWF HRP</b> in the micro ELISA plate wells
<b>Incubate for 1 hour at room temperature (18-25°C) (a)</b>		
<b>WS ELISA</b>	300µL	Proceed to 10 successive washings (b)
<b>TMB</b>	200µL	Immediately after the washing, introduce the substrate into the wells (b,c). <b>Nota:</b> The substrate distribution, raw by raw, must be accurate and at exact time intervals.
<b>Incubate for exactly 15 minutes at room temperature (18-25 °C) (a)</b>		
<b>Stop</b>	50µL	Following exactly the same time intervals, raw to raw, than for the addition of substrate, stop the colour development by introducing the 0.45M sulfuric acid (c).
<b>Wait for 10 minutes in order to allow the colour to stabilize then measure absorbance at 450 nm. Subtract the blank values (d).</b>		

Distribute calibrator dilutions, controls and specimens as rapidly as possible, in order to obtain homogeneous kinetics of the dosage. A too long delay (>10 min) between the first and the last distribution wells may have incidence on immunological kinetics and produce inaccurate results (underestimated value for the last wells).

- Avoid letting the plate in the bright sunlight during incubations and more particularly during colour development. A micro ELISA plate shaker can be used.
- Never let the plates empty between the addition of the reagents or following the washing step. The next reagent must be added within 3 minutes, in order to prevent the plate from drying, which could damage the immobilized components and reduce the reactivity plate. If necessary, keep the plate filled with Wash Solution and empty it just before the introduction of the next reagent. The washing instrument must be adjusted in order to wash the plates gently, and to avoid a too drastic emptying, which could damage coating and lower plate reactivity.
- For addition of the substrate, the time interval between each row must be accurate and exactly determined.
- For bichromatic readings, a reference wavelength at 620 nm or at 690 nm can be used.

## QUALITY CONTROL:

Using quality controls allows validating the method compliance, as well as the homogeneous of assays for a same lot of reagents.

Quality control plasmas must be included in each series, as per good laboratory practice, in order to validate test results. A new calibration curve must be carried out for each test series.

Each laboratory can establish acceptance ranges and verify expected performances in its analytical system.

## RESULTS:

- Obtained OD450 can vary according to the effective temperature during the assay run.
- Plot the calibration curve with the OD 450 nm along the Y-axis and the vWF concentration in %, along the X-axis by choosing the "best fit" interpolation mode (refer to the flyer in the kit).
- Results are expressed with the obtained OD450 for specimens and controls using the calibration curve.
- The concentration of vWF:CBA (%) in the test specimen is directly inferred from the calibration curve, when the standard dilution 1:50 is used.
- If other dilutions are used, the level obtained should be multiplied by the additional dilution factor used.
- Alternatively, a specific software (i.e., Dynex, Biolise, etc...) can be used for the calculation of concentrations.
- For **CI vWF** and **CII vWF**, the concentrations are directly deduced from the calibration curve.

**The results obtained should be for research use only and must not be used for patient diagnosis or treatment.**

## LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully.
- Any reagent presenting an unusual appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- If the washing step is not correctly performed, the negative control can produce a high absorbance value. In order to avoid non-specific color development, check that the washing step is performed efficiently.

## PERFORMANCES:

- Dynamic range: 0 to 150%.
- Detection threshold ≤ 5%.
- Intra-assay CV: ≤ 10 %.
- Inter-assay CV: ≤ 10 %.
- Interferences:** No interference was observed with the molecules and up to following concentrations:

Heparin	Bilirubin	Hemoglobin	Triglycerids
2 IU/mL	0.5 mg/mL	5 mg/mL	1.25 mg/mL

## REFERENCES:

- Luo GP. et al. von Willebrand Factor: more than a regulator of hemostasis and thrombosis. *Acta Haematol*, 2012.
- Peyvandi F. et al. Role of von Willebrand Factor in the haemostasis. *Blood Transfus*. 2011.
- Ruggeri Z.M. Von Willebrand factor, platelets and endothelial cells interactions. *J. Thromb. Haemost.* 2003.
- Kamphuisen P.W. et al. Familial clustering of factor VIII and von Willebrand factor levels. *Thromb Haemost.* 1998.
- CLSI Document GP44-A4: "Procedures for the handling and processing of blood specimens for common laboratory tests".
- CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008.

## SYMBOLS:

Symbols used and signs listed in the ISO 15223-1 standard, see Symbol definitions document.

**Stop** H290 : May be corrosive to metals.

**SD ELISA** **CD ELISA** **WS ELISA** H317 : May cause an allergic skin reaction.