

IMUCLONE™ vWF:CB ELISA

Product no. 59203

Storage: 2–8°C

For Research Use Only!

INTENDED USE

The IMUCLONE™ vWF:CB ELISA kit is an enzyme-linked immunosorbent assay for the differential diagnosis of von Willebrand Factor-Collagen Binding activity in human citrate plasma.

INTRODUCTION

Von Willebrand Factor (vWF) is a large, multi-functional glycoprotein with a key position in primary hemostasis. It exists as a multimer and has several functions: It mediates platelet aggregation via adhesion of the platelet membrane receptors following platelet activation.

In primary hemostasis it acts as a mediator in platelet adhesion to the subendothelium. Platelet aggregation is normally determined to characterize the adhering properties (parameter: Ristocetin-dependent platelet aggregation).

However, it does not reflect the physiological state and function of vWF. The binding of vWF to collagen is used as a tool for measuring the adherent properties of vWF [1, 2].

EXPLANATION OF THE TEST

The vWF:CB ELISA is a 2-step-ELISA. The microtiter plate wells are coated with human collagen type III. Diluted citrate plasma samples are pipetted into the wells and incubated. During incubation vWF binds to the collagen. Unspecific binding is removed by washing steps, followed by a second incubation step with conjugate. Anti-vWF-peroxidase conjugate reacts with bound vWF. Excess conjugate is removed through a washing step and the amount of vWF in the plasma sample is quantified using the peroxidase reaction and a standard curve.

REAGENTS AND MATERIAL

MTP, Microassay Plate, 96 wells with 12 x 8 well strips coated with human collagen type III in resealable aluminum bag. Ready-to-use.

CAL 1, CAL 2, CAL 3, CAL 4, CAL 5, CAL 6, 6 vials calibrators 1-6, lyophilised human plasma with stabiliser and preservative. The concentration of vWF in the vials is lot-specific and printed on the labels. Reconstitute before use!

POS LL, POS HL, control plasma, LL, "Low level", HL, "High Level" for quality control, lyophilised, with stabiliser and preservative. The concentration of vWF in the vials is lot-specific and printed on the labels. Reconstitute before use!

WASH 10x, Wash Solution Concentrate (10x), 1 bottle, 100 mL; contains 0.01% (w/v) Thimerosal. Dilute before use!

DIL, Sample Buffer, 1 bottle, 100 mL, colored red, contains 0.005% (w/v) Thimerosal. Ready-to-use.

CON 20x, Conjugate 20x, 1 vial polyclonal (rabbit) anti-vWF conjugated to peroxidase, colored blue, 0.75 mL. Dilute immediately before use!

S 20x, Substrate (20x); 1 vial, 2.5 mL TMB concentrate. Dilute immediately before use!

STOP, Stop Solution, 1 vial, 15 mL, contains 0.5 M H₂SO₄. Ready-to-use!

2 Adhesive foils
1 Pipetting scheme

Material Required but not Provided

Timer
Test tubes for sample dilution
Graduated cylinder (1000 mL)
Precision pipettes (5, 20, 100, 500 and 1000 µL)
Pipettes (10 and 20 mL)
Multichannel and dispensing pipettes (50 and 200 µL)
Sample mixer
Distilled water
Gloves
ELISA reader, 450 nm filter

REAGENT PREPARATION AND STABILITY

Specimen Collection

Handle and dispose of all specimens as if they are capable of transmitting infectious agents.

Sample collection is critical and should be handled by experienced laboratory personal only! To prepare plasma, blood samples should be collected with sodium citrate as anticoagulant and should be centrifuged immediately for 15 min at 2000 x g. This entire procedure must be completed quickly.

Preparation of Reagents

Allow kit components to reach room temperature (20-26°C).

1. Reconstitute calibrators 1-6, and controls: Dissolve content of each vial with 200 µl **sample buffer (DIL)**, incubate for 15 min., vortex for 10 sec. After reconstitution the contents of calibrators and controls should be clear or slightly turbid.

2. Dilution of Calibrators, Controls and Samples, 1+40: e.g. pipet 10 µl calibrator, control or sample in 400 µl sample buffer and mix.

3. Anti-vWF Conjugate, 1+20: Example for 1 strip with 8 wells: dilute 50 µl conjugate in 1000 µl **sample buffer**. As the diluted solution is not stable, it is recommended to always prepare the required amount of conjugate dilution only.

4. Wash Buffer, 1+9: Example for 1 strip with 8 wells: Dilute 5 ml wash buffer concentrate in 45 ml **dist. water**. Mix well!

5. Substrate Solution, 1+19: Example for 8 wells: Dilute 100 µl substrate in 1900 µl **dist. water**. Always use clean vials and pipets, do not use a magnetic bar and avoid contact with metal!

Stability

All unopened kit components are stable until the expiry date indicated. After opening reagents are stable as follows:

Component	Form	Temperature	Stability
Wash buffer	undiluted	2-8°C	6 months
Calibrators, Controls	reconstituted	-20°C	6 months
	diluted		Use immediately
Conjugate	undiluted	2-8°C	6 months
	diluted	20-26°C	60 min.
Substrate	undiluted	2-8°C	6 months
	diluted	20-26°C	30 min.

ASSAY PROCEDURE

1. Sample Incubation: Pipet **100 µl** diluted calibrators / controls / samples into appropriate wells and cover with foil. Incubate at **room temperature (20-26°C) for 60 min.**

2. Wash: Empty microassay strips and fill each well with at least **250 µl** of ready-to-use wash buffer. Empty wells and repeat this wash step twice. Remove excess liquid by tapping the strips onto absorbent paper.

3. Conjugate Incubation: Pipet **100 µl** of the diluted conjugate into each well, cover with foil and incubate **60 min at room temperature.**

4. Wash: Empty microassay strips and fill each well with at least **250 µl** of ready-to-use wash buffer. Empty wells and repeat this wash step twice. Remove excess liquid by tapping the strips onto absorbent paper.

5. Substrate Reaction: Pipet **100 µl** ready-to-use substrate solution into each well and incubate for **15 min at room temperature.**

6. Stop Reaction: Pipet **100 µl** stop solution into each well. Shake for **10 sec** and measure colour reaction within **30 min** at **450 nm** (reference wave length **620-690 nm**).

IMPORTANT NOTES

For professional use only!

Do not interchange reagents from kits with different lot numbers. Hemolytic, lipemic, icteric or microbial contaminated samples may cause false results.

Keep incubation temperature at 20-26°C.

Always adhere to the same pipetting scheme.

Double determinations are recommended.

Do not vary the incubation time more than ±10%. The time starts after pipetting the last sample.

Time for pipetting the samples: maximum 60 sec per strip.

Time for pipetting conjugate-, substrate and stop solution: maximum 10 sec per strip.

PRECAUTIONS

Stop solution (contains 0.5 M H₂SO₄) and Substrate may cause skin irritations. If acid or TMB should come into contact with eyes, rinse it out immediately with plenty of water and consult a physician. Calibrators and Controls have been tested for HBsAg, HIV and HCV antibodies and found to be negative. However, all human blood products should be considered potentially infectious. Some reagents contain Thimerosal. Do not swallow! Avoid contact with skin or mucous membranes!

RESULTS

Creating a Standard Curve

Calculate mean values for calibrators, controls and samples. Create a standard curve by plotting the OD readings (y-axis) against the activity values of the calibrators (x-axis).

It is recommended to use a 4-parameter curve fit for calculating results. Most software is programmed for this purpose and may be used to determine the activity of vWF collagen binding (see equation 1).

Equation 1:
$$Y = d + (a-d)/(1 + (x/b)^b)$$

Validation of Results

The absorption of the highest calibrator should be between OD 1.0 and OD 2.5. The values of the controls must be found within the range printed on the label.

The control values obtained are used to check whether the evaluation is correct.

In case of any deviation the following technical issues should be checked:

- expiration dates of (prepared) reagents
- storage conditions
- pipettes and devices
- incubation temperature
- washing methods
- timer
- photometer

Calculation of Results

The concentration of the samples can be read directly from the standard curve.

Samples with an absorbance exceeding that of the highest calibrator should be prediluted with dilution buffer 1+1. Consequently, the obtained values have to be multiplied by a factor 2.

Interpretation of Results

Determination of von Willebrand factor activity is required for diagnosis and classification of von Willebrand disease. In addition, von Willebrand factor activity can be of prognostic relevance in several clinical entities including thromboembolic and cardiovascular disorders in which elevated activity correlates with a poor prognosis. The results obtained with the vWF:CB ELISA should be interpreted in relation to von Willebrand factor activities determined by established procedures, e.g. ristocetin cofactor, electrophoresis, vWF antigen quantification by ELISA. Furthermore the clinical background of the patients as well as a potential factor VIII deficiency should be considered. A strategy for differential diagnosis has been described by Fischer and Thomas (cf ref. 1)

The obtained results in U/mL can be converted into %: 1 U/mL corresponds to 100%.

Calibration

The calibrators of the test were calibrated using the WHO Standard (NIBSC Code 97/589).

REFERENCES

- (1) Fischer, B. E., Thomas, K. B., Dorner, F.: Collagen covalently immobilized onto plastic surfaces simplifies measurement of Willebrand factor-collagen binding activity. *Ann. Hematol.* **76**, 159-166 (1998)
- (2) Siekmann, J., Turecek, P. L., Schwarz, H. P.: The determination of von Willebrand factor activity by collagen binding assay. *Haemophilia.* **4**, 15-24 (1998).