TECHNOZYM® vWF:CBA ELISA Collagen Typ VI
For research use only

symbols key

- **DIL**: dilute or dissolve in
- **LOT**: lot
- **MTP**: microtiter plate
- **REF**: catalogue number
- **RTU**: ready to use
- **RUO**: research use only
- **SUB**: substrate
- **STOP**: stop solution
- **WASH**: washing solution concentrate

REF 5450321 TECHNOZYM® vWF:CBA ELISA Collagen Typ VI

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For Research Use Only
PRODUCT DESCRIPTION

INTENDED USE

The von Willebrand Factor (vWF) is a large, multifunctional glycoprotein, occupying a key position in primary haemostasis. It has a multiple structure with several functions:

- It is the carrier protein for Factor VIII in plasma; it forms a complex with it. This protects Factor VIII from early proteolytic decomposition.
- It acts as a mediator for platelet aggregation by attaching itself to platelet membrane receptors (GP Ib and GP I/IIa) following previous platelet activation.
- It plays a part in primary haemostasis by acting as a mediator between adhered platelets and the subendothelium (lesioned vascular wall).

In order to analyze the adhesive properties, as a rule the platelet aggregation is measured. The binding capacity of the vWF, its binding capacity to collagen serves as a parameter which corresponds to the physiological function of the vWF.

COMPOSITION

1. ELISA test strips (12) with 8 wells each, coated with human collagen Typ VI; the drying agent is supplied in an aluminum bag.
2. Washing buffer concentrate: (PBS; pH 7.3); containing detergent; 0.01% merthiolate; 1 bottle, 80 mL
3. Incubation buffer: (PBS; pH 7.3); contains stabiliser protein; 0.05% proclin; and dye 1 bottle, 90 mL, ready to use.
4. Calibrators (Standards) numbered; lyophilised; 1 bottle each.
5. Concentrations are lot-dependent; consult label on the vial.
6. Chronogen TMB (tetramethylbenzidine); 1 bottle, 12 mL; ready to use.
7. Chromogen TMB (tetramethylbenzidine); 1, 20 mL; ready to use.
8. Stopping solution: sulphuric acid 0.45 mol/L; 1 bottle 12 mL; ready for use.

MATERIAL REQUIRED (but not supplied with the kit)

1. Distilled water
2. Test tubes for diluting standard and samples
3. Measuring cylinder (1000 mL)
4. Precision pipettes (10, 100 and 1000 µL)
5. Variable pipette (1000 µl)
6. Multichannel and/or dispensing pipettes (100 and 200 µL)
7. ELISA washer or multichannel pipette
8. ELISA reader with 450 nm filter, with a 620 nm reference filter if available.

WARNING AND PRECAUTIONS

- For research use only.
- All human blood or plasma products as well as samples must be considered as potentially infectious. They have to be handled with appropriate care and in strict observance of safety regulations. The rules pertaining to disposal are the same as applied to disposing hospital waste.
- Calibrators and control plasma are made from human blood and any individual plasma involved in the procedure is HBsAg, HIV 1/2 Ab and HCV-Ab-negative (see labels on kit and/or bottles).
- Stopping solution (sulphuric acid) may irritate the skin. Should acid get into your eyes, rinse immediately with water and consult a doctor.
- The reagents sometimes contain preserving agents (merthiolate). Beware of swallowing!
- Avoid contact with skin or mucous membranes.

STABILITY AND STORAGE

All components contained in the kit may be used until the expiry date indicated. The bench stability of the components after opening, reconstitution and/or dilution may be inferred from the data below:

When necessary the samples, controls and calibrators can be frozen/thawed up to 5 times. But making aliquots is recommended.

TEST PROCEDURE PREPARATION OF SAMPLES

Material: plasma.

Obtaining plasma: mix 9 parts venous blood with 1 part sodium citrate solution (0.11 mol/L) and centrifuge for 15 minutes at a maximum of 2500 g (DIN 58805). The plasma sample may be stored for 3 hours at room temperature; otherwise the sample ought to be frozen immediately after centrifugation. Stable at -20°C for 6 months.

PREPARATION OF REAGENT

1. Before starting the test, all the required components are to be brought to room temperature.
2. Preparing the washing buffer: Dilute 1 part by volume washing buffer concentrate with 11.5 parts by volume distilled water (1+11.5). Mix well! (Diluted washing buffer concentrate = washing buffer). There may be crystalline precipitations which will dissolve at 37°C within 10 minutes.
3. Reconstituting calibrators and control plasma:
- Calibrators and control plasma are reconstituted with 500 µL distilled water and mixed for 10 seconds after a reconstitution time of 15 minutes (vortex mixer). Reconstituted components are clear to slightly turbid.
- Diluting calibrators, control plasma and samples (1+40): Dilute 10 µL samples, 10 µL calibrators and/or10 µL controls with 400 µL each of incubation buffer. Mix for 10 seconds! 
5. Preparing the conjugate working solution (1+50): Dilute 1 part by volume conjugate with 50 parts by volume incubation buffer.

For 8 test wells: Mix 20 µL conjugate with 1000 µL incubation buffer.

PERFORMANCE OF THE TEST

SAMPLE INCUBATION (reference 1, 2) diluted calibrators diluted control plasma diluted samples into test wells. cover test strips with film. incubate at room temperature 45 minutes

WASHING (reference 1, 3, 4) washing buffer 3 x 200 µL incubate at room temperature 45 minutes

CONJUGATE REACTION (reference 1, 2) pipette conjugate working solution into wells, cover test strip with film. incubate at room temperature 15 minutes

SUBSTRATE SOLUTION (reference 1, 3, 4) washing buffer 3 x 200 µL incubate at room temperature 45 minutes

STOP SOLUTION (reference 5) pipette stopping solution into wells 100 µL incubate at room temperature 15 minutes

MEASURING (reference 5) ELISA-Reader, 450 nm Measure within 10 minutes

Room temperature is 20 ... 25°C

References

1. Reagents of different lots must not be combined. 2. Precision and performance, among others, essentially depend on the following factors: Thorough mixing of all substances used for dilution. Test calibrators, controls and samples in duplicates. Incubation to be done at correct temperatures. Strict observance of the order of pipetting and of the time element as indicated. The time for sample incubation, conjugate and substrate reaction as indicated starts after pipetting the last sample. Incubation times should not vary by more than ±10%. During sample incubation and conjugate reaction, the time for pipetting the diluted calibrators/samples/control plasma and/or conjugate solutions must not exceed 30 seconds per ELISA test strip (8 wells). During substrate reaction and at stopping, the time needed for pipetting the substrate and/or the stopping solution must not exceed 10 seconds per ELISA test strip. Short pipetting times may be secured by using multichannel- and dispensing pipettes. Label/number strips with a water-resistant pen in case the strips accidentally fall out of the frame during testing.

ASSESSMENT OF REFERENCE CURVE

8 hours
6 months
EXPRESS: 20°C
6 months
3 weeks
30 minutes
room temperature
10 minutes
calibrators/samples/control plasma and/or conjugate solutions must not exceed 60 seconds per ELISA test strip (8 wells).

LEADING OF THE TEST

Reduced levels of vWF:CBA are associated with blood group 0.

vWF:CBA is also affected by physical exercise, pregnancy, use of contraceptive pill, ethnic group and the antigen increases with age.

CALCULATION OF THE RESULTS

Calculation formula of levels of vWF:CBA: Reduced levels of vWF:CBA are associated with blood group 0.

CALCULATION OF THE RESULTS

The validity of the test may be checked on the basis of the calculated control values. Example of standard curve.

Setting up a reference curve: X axis: Concentration vWF:CBA U/ml (1U/ml = 100%) Y axis: Extinction Graph plot is linear-linear with a linear or cubic spline

REFERENCE Ranges Normal range for vWF:CBA between 0.6 – 1.3 U/ml (60 – 130%). It is recommended that individual laboratories establish their own normal range.

STANDARDIZATION

The calibration material used is the WHO International Standard for Blood coagulation Factor VIII and von Willebrand factor in plasma (human).

LITERATURE

1) [reference](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2006894/)
2) [reference](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2006894/)
4) [reference](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2006894/)
5) [reference](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2006894/) Detection of VWD and discrimination of VWD Subtypes, Depends on Collagen Source. E J Favaloro