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TECHNOZYM® vWF:CBA ELISA Collagen Typ I

For research use only

GB

REF 5450311 TECHNOZYM® vWF:CBA ELISA Collagen Typ I

Symbols key			
	Manufacturer		Expiry date
	Storage temperature		Consult instructions for use
AQUA	Distilled water		Determinations
BUF	Reaction buffer	LOT	Lot
CAL	Calibrator	MTP	Microtiter plate
CONJ	Conjugate	REF	Catalogue number
CONT	Control	RTU	Ready to use
DIL	Dilute or dissolve in	STOP	Stop solution
INC	Incubation buffer	SUB	Substrate
RUO	For resarch use only	WASH	Washing solution concentrate

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TECHNOZYM® vWF:CBA ELISA Collagen Typ I

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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 and thus 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 IIb/IIIa) following previous platelet activation.
- It plays a part in primary haemostasis by acting as a mediator between adhesioned platelets and the subendothelium (lesioned vascular wall).

In order to analyze the adhesive properties, as a rule the platelet aggregation is measured (measuring system = ristocetin-dependent platelet aggregation).
However, this does not reflect the physiological setting nor the function of the vWF. For determining the adhesive properties 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 I; the drying agent is supplied in an aluminium 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 for use.
4. Calibrators (Standards) numbered; lyophilised; 1 bottle each. **Concentrations are lot-dependent; consult label on the vial.**
5. Control plasmas "low level" and "high level" for checking purposes, lyophilised; 1 bottle each. **Concentrations are lot-dependent; consult the label on the vial.**
6. Conjugate polyclonal Anti-vWF-POX; dyed blue; 1 bottle, 0.3 mL.
7. Chromogen TMB (tetramethylbenzidine); 1 bottle, 12 mL; ready to use.
8. Stopping solution: sulphuric acid 0.45 mol/L; 1 bottle 12 mL; ready for use.
9. Adhesive film: for ELISA test strips (2).

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 plasmas 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, wash out 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 as indicated. The bench stability of the components after opening, reconstitution and/or dilution may be inferred from the table below:
When necessary the samples, controls and calibrators can be frozen/thawed up to 5 times. But making aliquots is recommended.

Material/Reagent	State	Storage	Stability
Calibrators, control plasmas	after reconstitution	room temperature -20°C	8 hours 6 months
ELISA test strip	after opening	2 ... 8 °C with adhesive film in plastic bag with drying agent	expiry date
Washing buffer concentrate	after opening	2 ...8°C	6 months
Washing buffer	1+11.5 dilution of concentrate	2 ... 8 °C	3 weeks
Incubation buffer	after opening	2 ... 8 °C	2 months
Conjugate	after opening	2 ... 8 °C	6 months
	working solution	room temperature	60 minutes
Chromogen TMB	after opening	2 ... 8 °C	expiry date

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 minimum of 2500 g (DIN 58905). 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 plasmas:
   Calibrators and control plasmas 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.
4. Diluting calibrators, control plasmas and samples (1+100): Dilute 5 µL samples, 5 µL calibrators and/or 5 µL controls with 500 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 plasmas** **diluted samples** into test wells. cover test strips with film.	**100 µL**
	incubate at **room temperature**	**45 minutes**
WASHING (reference 1,3,4)	**washing buffer**	**3 x 200 µL**
CONJUGATE REACTION (reference 1,2)	pipette **conjugate** working solution into wells, cover test strip with film	**100 µL**
	incubate at **room temperature**	**45 minutes**
WASHING (reference 1,3,4)	**washing buffer**	**3 x 200 µL**
SUBSTRATE REACTION (reference 1,2)	pipette **substrate solution** into test wells cover test strips with film	**100 µL**
	Incubate at **room temperature**	**25 minutes**
STOP SOLUTION (reference 1,2)	pipette **stopping solution** into wells	**100 µL**
MEASURING (reference 5)	ELISA-Reader, **450 nm**	Shake 10 sec., 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 plasmas and/or conjugate solutions must not exceed 60 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.

3. Label/number strips with a water resistant pen in case the strips accidentally fall out of the frame during testing.
4. After the last washing, wells must be aspirated thoroughly, turned upside down and positioned on a blotting paper; by gentle tapping, the last remnants must be removed.
5. Measuring the difference in wave lengths at 450 and 620 nm or at 450 and 690 nm, the precision of the test is increased.

LIMITATION 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.

ANALYSIS RESULTS

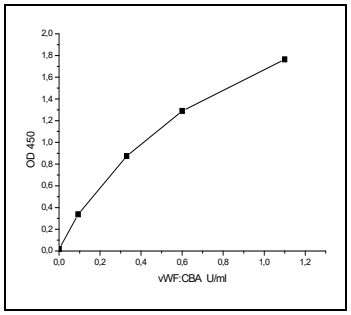
CALCULATION OF THE RESULTS

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

Assessment of reference curve

- The extinction coefficient of the highest calibrator should be between 1.0 and 2.5.
- The validity of the test may be checked on the basis of the calculated control values.

Example of standard curve.



Measuring concentration of samples

- Read off the concentration from the reference curve.
- If there are samples with extinction coefficients higher than that of the highest point on the curve, they have to be prediluted with incubation buffer (1+1). The measured concentration then has to be multiplied with the dilution factor 2.

REFERENCE RANGE

Normal range for vWF:CBA is 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) Blood 69; 1691 – 1695, 1987. The effect of ABO blood group on the diagnosis of vWD. Gill et al.
2) Thromb Haemost 2000; 83: 127 – 35. Collagen Binding Assay for von Willebrand Factor (VWF:CBA) Detection of VWD and discrimination of VWD Subtypes, Depends on Collagen Source. E J Favaloro
3) Haemophilia (Suppl. 3), 1998, 15 – 24. The determination of von Willebrand factor activity by collagen binding assay. Siekmann et al.