REF 5006011 Ceveron® TGA RB Kit

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RUO

Ceveron® TGA RB Kit - English

INTENDED USE

Ceveron® TGA RB kit is used for determination of thrombin generation in human citrated plasma on Ceveron® alpha TGA. Ceveron® TGA RB trigger reagent is used for measurement of bleeding tendency, for monitoring therapy in heamophilia samples and for monitoring FVIII inhibitor bypassing therapy.

SUMMARY

Ceveron® TGA RB is based on monitoring the fluorescence generated by the cleavage of a fluorogenic substrate by thrombin over time, upon activation of the coagulation cascade in plasma by Ceveron® TGA RB trigger composed of tissue factor and negatively charged phospholipids.

The trigger composition is specially adapted to detect very sensitive changes in the positive feedback loop of intrinsic pathway activation upon activation of the extrinsic pathway, giving the assay high sensitivity to changes in the low range of FVIII and FIX levels.

From the changes in fluorescence over time, the concentration of thrombin (nM) in the sample can be calculated using the respective thrombin calibration curve. The increase in thrombin concentration over time allows the calculation of the thrombin generation curve and to calculate thrombin generation parameters.

REAGENTS

The Ceveron® TGA RB Kit contains:

mL	Reagent	Description
2 x 1	Ceveron® TGA RB	Trigger reagent with low concentration of phospholipid micelles containing rhTF in Tris-Hepes-NaCl buffer, lyophilized.
2 x 1.5	Ceveron® TGA BUF	Tris-Hepes-NaCl buffer, lyophilized.
2 x 3	Ceveron® TGA SUB	Fluorogenic substrate 1 mM Z-G-G-R-AMC, lyophilized.
2 x 1	Ceveron® TGA CONT H	Human plasma with increased thrombin generation, lyophilized.
2 x 1	Ceveron® TGA CONT L	Human plasma with decreased thrombin generation, lyophilized.
1 x 25	Calcium Chloride solution 25 mM	$CaCl_2$ 25 mM, ready to use.

Material required (not supplied with the kit)

- Pipettes
- Distilled water

REF 5006347 Ceveron® TGA CAL Set

Warning and precautions

RUO – for research use only.

Every single donor plasma and every lot of the controls included is tested and found negative for HbSAg, HIV 1/2 antibodies and HCV antibodies. However, general precautions should be taken by handling all human source materials as potentially infectious.

A Material Safety Data Sheet for this product is available from www.technoclone.com

Stability and storage

The expiry date printed on the labels is only applicable to storage of the unopened containers at 2…8°C. Stability after reconstitution:

Reagent	Ceveron® alpha TGA	2...8°C
Ceveron® TGA RB	8 hours	5 days
Ceveron® TGA BUF	8 hours	5 days
Ceveron® TGA SUB	8 hours	5 days
Ceveron® TGA CONT H	4 hours	-
Ceveron® TGA CONT L	4 hours	-
$CaCl_2$ 25 mM	7 days	4 weeks

Avoid contamination by microorganisms.

Plasmas should be frozen only once; during storage, the vials should be tightly capped.

Stability of the sample material:

Sample material	18...25°C	-20°C
Platelet poor plasma	4 hours	1 month

An immediate centrifugation after blood withdrawal is recommended. Further we recommend an immediate shock freezing of the centrifuged samples.

Attention! The frozen samples should be stored in a constant environment - avoid exposing the samples to variations in temperature. Before transportation we recommend to centrifuge and prepare the plasma samples.

TEST PROCEDURE

Preparation of samples

For Platelett poor plasma (PPP) separation, mix 9 parts of venous blood and 1 part sodium citrate solution (0.11 mol/L) and centrifuge for 15 minutes at a RCF of at least 2.500 x g (corresponding to DIN 58905).

Preparation of reagents

All lyophilized reagents of the Ceveron® TGA reagent kit must be dissolved in the volume of distilled water indicated on the vials. Reconstitution time is 20 minutes for the reagents and controls.

Vials with lyophilized reagents and distilled water used for reconstitution need to reach room temperature (18…25°C) before reconstitution.

Vials have to be mixed thoroughly to ensure that the whole material is resuspended. Mixing is performed best by careful upside-down movements of the vial. Vortex must be avoided as it would cause air bubbles in the reagent and these would disturb fluorescence measurement.

Special care has to be taken on substrate reconstitution. The lyophilized material is clear and can adhere to the wall of the vial. Make sure that the whole material is dissolved!

Before using the reagents, the vials need to be mixed again thoroughly by careful upside-down movements. Vortex must be avoided.

All reconstituted stored reagents should reach room temperature before use.

The validity of the test is checked by determination of controls. Please consult the lot specific batch table included in the kit for the reference ranges.

Performance of the test

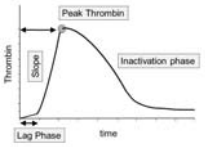
The Ceveron® TGA RB Kit is always used in combination with the Ceveron® TGA CAL Set.

Technoclone provides application sheets for Ceveron® alpha TGA. The application sheets contain analyser/assay specific handling and performance information. Please consult the instruction manual of the Ceveron® alpha.

To establish a calibration curve on Ceveron® alpha TGA use the Ceveron® TGA CAL Set. Follow the instructions from kit insert and from application sheet for Ceveron® alpha TGA.

Analysis of samples

The Ceveron® alpha TGA software calculates thrombin generation in the sample over time and the results are given in nM thrombin generated in the sample for each point of time during the whole coagulation process. The pattern seen resembles the figure provided. The following parameters can be used as readout:



- **Lag phase** from the time point when the TGA reagent including $CaCl_2$ is added until the first burst in thrombin formation
- **Peak thrombin:** Maximal concentration of thrombin formed
- **AUC:** Area under the curve

Normal range

Following normal ranges were determined testing 100 healthy normal donor PPP samples:

Reagent	nM Peak Thrombin	nM AUC
Ceveron® TGA RB Kit	43 – 368	1236 - 2945

It is recommended that each laboratory establish and controls its normal range. The evaluation and interpretation of sample results has to be made by specialized staff and the s medical history has to be considered.

LIMITATION OF THE TEST

Reliable results can only be obtained when blood collection is standardized and follows the criteria of minimal activation of the clotting system during venipuncture. Care has to be taken during centrifugation of blood and plasma that only such plasma samples are used for the assays that comply with the requirements for the respective assays. In case of use of incorrect plasma samples interpretation of the results might become impossible. α2-MG-thrombin complexes formed during thrombin generation reaction do not influence the most important TGA parameter Peak Thrombin, but can lead to increase of AUC values. All types of anticoagulants influence thrombin generation parameters. Microparticles of different origin trigger thrombin generation, influencing the TGA parameters. Care has to be taken to avoid microparticle release during sample preparation and storage.

STANDARDISATION

The thrombin calibrator is calibrated against the Thrombin Reference Preparation of the WHO (see batch table).

LITERATURE

Please contact Technoclone www.technoclone.com or your local distributor.

 manufacturer

 storage temperature

 expiry date

 consult instructions for use

 Biological risk

RUO Research use only

 Determinations

LOT lot

REF catalogue number

GTIN Global Trade Item Number

BUF Buffer

SUB Substrate

CONT Control

Technoclone Herstellung von Diagnostika und Arzneimitteln GmbH, Brunner Strasse 67, 1230 Vienna, Austria

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