REF **5006015 Ceveron TGA RC High** ENG

3013828RUO Rev.002 26/09/2021 RUO

Ceveron TGA RC High - English

INTENDED USE

Ceveron TGA RC High is used for determination of thrombin generation in human citrated plasma on Ceveron alpha TGA and capable TGA measuring instruments of the Ceveron 100 series.

Ceveron TGA RC High trigger reagent is used for measurement of thrombin generation in anticoagulated human plasma samples.

SUMMARY

Ceveron TGA RC High 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 RC High trigger composed of tissue factor and negatively charged phospholipids.

The trigger composition is specially adapted to induce a thrombin burst upon activation of the extrinsic pathway, which allows the measurement of thrombin generation in anticoagulated samples.

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 RC High contains:

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

Material required (not supplied with the kit)

- Distilled water
- Precision pipettes
- Variable pipette
- Laboratory timer
- REF 5006347 Ceveron TGA CAL

Warning and precautions

- RUO for research use only.
- This kit is intended for use by personnel trained in laboratory procedures and universal precautions for the use of chemicals and potentially biohazardous substances.
- 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.
- Control plasmas are made from human blood and any individual plasma involved in the procedure is tested HbsAg, HIV 1/2 Ab and HCV-Ab-negative by FDA approved or CE marked methods. However, all human blood products should be handled as potentially infectious material.
- Get a Material Safety Data Sheet for this product 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 opened/ in use:

Reagent	Ceveron alpha TGA / Ceveron 100 series (open vial)
Ceveron TGA RC High	8 hours
Ceveron TGA BUF	8 hours
Ceveron TGA SUB	8 hours
Ceveron TGA CON H	4 hours
Ceveron TGA CON L	4 hours
$CaCl_2$ 25 mM	7 days

Avoid contamination by microorganisms.

TEST PROCEDURE

Preparation of plasma samples

For preparation of Platelet Poor Plasma samples a standardized procedure such as CLSI H21-A5 or DIN 58905 is required to be implemented to minimize variability caused by preanalytical steps.

It is recommended to use the locally established sample collection method to reduce additional preanalytical errors.

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.

Thaw frozen samples rapidly at 37 °C. Gently mix before testing. After thawing, the assay must be performed within 2 hours.

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

Avoid contamination by microorganisms.

Preparation of reagents

Before starting the test, all the required components must be brought to room temperature.

Avoid foam formation when reconstituting plasmas and mixing reagents or buffers.

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

- *Ceveron TGA RC High:* Dissolve each bottle of lyophilized TGA RC High trigger in 1.0 mL distilled water and swirl gently. Allow the reconstituted material to stand for 20 minutes at room temperature before use.
- *Ceveron TGA BUF:* Dissolve each bottle of lyophilized buffer in 1.5 mL distilled water and swirl gently. Allow the reconstituted material to stand for 20 minutes at room temperature before use.
- *Ceveron TGA SUB:* Dissolve each bottle of lyophilized substrate in 3.0 mL distilled water and swirl gently. Allow the reconstituted material to stand for 20 minutes at room temperature before use.
- *Ceveron TGA CON H:* Dissolve each bottle of lyophilized control high in 1.0 mL distilled water and swirl gently. Allow the reconstituted material to stand for 20 minutes at room temperature before use.
- *Ceveron TGA CON L:* Dissolve each bottle of lyophilized control low in 1.0 mL distilled water and swirl gently. Allow the reconstituted material to stand for 20 minutes at room temperature before use.
- *Calcium Chloride solution:* Ready to use.

Performance of the test

The Ceveron TGA RC High is always used in combination with the Ceveron TGA CAL.

Ceveron TGA RC High is performed on the Ceveron alpha TGA, the Ceveron t100 and the Ceveron s100 with the respective application.

Ceveron TGA RC High is calibrated on the Ceveron alpha TGA, the Ceveron t100 and the Ceveron s100 using the Ceveron TGA CAL. Follow the instructions from kit the Ceveron TGA CAL insert to perform thae calibration.

Ceveron TGA controls low and high are recommended for a complete quality control program. Ceveron TGA controls low and high are designed for this program. Each laboratory should establish its own mean and standard deviation for a quality control program in order to monitor laboratory testing. Controls should be analyzed before validating sample results in accordance with good laboratory practice.

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.

INTERPRETATION OF RESULTS

Ceveron TGA RC High results are reported in nM Peak Thrombin.

The results can also be displayed in Lag Phase, slope and area under the curve (AUC).

The Ceveron 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

REFERENCE RANGE

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

Normal range for Ceveron TGA RC High Kit: 84 – 626 nM Peak Thrombin

Normal range for Ceveron TGA RC High Kit: 1299 – 3145 nM AUC

It is recommended that individual laboratories establish their own normal range.

STANDARDISATION

The thrombin calibrator is calibrated against the Thrombin Reference Preparation of the WHO.

LITERATURE

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

EDITORIAL NOTE

This document is available in several languages. The translations have been done using the master document in English. In the event of doubts or discrepancies, the wording in the master document in English shall take precedence.


Manufacturer


Storage temperature


Expiry date


Consult instructions for use


Biological risk

CONT Control


Determinations

RUO Research use only

LOT Lot

REF Catalogue number

GTIN Global Trade Item Number

BUF Buffer

SUB Substrate

Technoclone Herstellung von Diagnostika und Arzneimitteln GmbH, Brunner Str. 67 - 1230 Vienna, Austria