REF **5800100** **TECHNOFLUOR ADAMTS13 Activity** ENG

3050525RUO Rev.002 23/11/2021 RUO

TECHNOFLUOR ADAMTS13 Activity - English

INTENDED USE

ADAMTS13 activity assay based on a fluorogenic VWF substrate to test automated the quantitative determination of ADAMTS13 activity in citrated human plasma, for use in research activities on the Ceveron s100.

SUMMARY

Von Willebrand factor (VWF) promotes clot formation by tethering platelets at the site of vessel injury and can also contribute to platelet aggregation. It is comprised of subunits of differing sizes, termed multimers, whose adhesiveness increases with size. The enzyme ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type 1 motif 13) acts as a gatekeeper against generation of highly thrombogenic ultra large multimers by regulating multimer size via a specific cleavage site, thereby breaking down large multimers into smaller, less reactive forms. Ultra large multimers can achieve millimeters in length if unregulated. In ADAMTS13 deficiency states, unusually large hyperfunctional VWF multimers can accumulate, leading to microvascular thrombosis and organ damage/failure due to VWF-platelet aggregates. In turn, a microangiopathic haemolytic anaemia can ensue, red blood cells being sheared as they travel past and through the microthrombi. ADAMTS13 deficiency can be hereditary or acquired, the latter commonly due to ADAMTS13 autoantibodies, and is termed thrombotic thrombocytopenic purpura.

In the TECHNOFLUOR assay, ADAMTS13 from the plasma sample cleaves a VWF73 based substrate, thereby releasing the dark quencher. Consequently, the energy transfer that quenches fluorescence from an adjacent emitter does not occur, allowing emission of fluorescence. The fluorescent signal is proportional to the ADAMTS13 activity in the sample.

REAGENTS

The TECHNOFLUOR ADAMTS13 Activity contains:

	Reagent / Content	Description
2 x 1 mL	Substrate (SUB)	ADAMTS13 specific substrate with dark quencher
2 x 3 mL	Buffer (BUF)	ADAMTS13 buffer for sample dilution

Material required (not supplied with the kit)

- Distilled water
- Precision pipettes
- Calibration Plasma*
  - REF 5220110 Coagulation Reference 5 x 1 mL
  - REF 5800102 TECHNOFLUOR ADAMTS13 Activity 0 CAL 2 x 1 mL
- Control Plasma*
  - REF 5020040 Coagulation Control N 5 x 1 mL
  - REF 5021055 Coagulation Control A 5 x 1 mL
- Laboratory timer

* or any other package sizes.

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 must be applied.
- All human blood or plasma products as well as test 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.
- 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 / Content	Ceveron s100 (open vial)	2…8 °C (closed vial)	< -20 °C (closed vial)
Substrate (SUB)	3 days	1 month	2 months
Buffer (BUF)	3 days	2 months	Do not freeze

Substrate should only be frozen once. Thawing must be performed rapidly in a water bath kept at 37 °C.

TEST PROCEDURE

Preparation of plasma samples

Collect nine parts of freshly drawn venous blood in one part trisodium citrate (3.2 %). Refer to CLSI Document H21-A5 for instructions on specimen collection, handling, and storage.

Thaw frozen samples rapidly at 37 °C and centrifuge and separate if necessary. Gently mix before testing. After thawing, the assay must be performed within 2 hours. Samples may be frozen once at <-70 °C.

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.

- *Substrate:* Dissolve each bottle of lyophilized substrate in 1.0 mL distilled water and swirl gently. Allow the reconstituted material to stand for 10 minutes at room temperature before use. For standardizing tests, a reconstitution time of 30 minutes is recommended. Swirl to mix before use.
- *Buffer:* The ADAMTS13 buffer is ready to use.

Performance of the test

The TECHNOFLUOR ADAMTS13 Activity is performed on the Ceveron s100 with the respective application.

Calibration is performed using a serial dilution of Coagulation Reference in TECHNOFLUOR ADAMTS13 Activity 0 CAL. Normal and abnormal controls are recommended for a complete quality control program. Coagulation Control N and Coagulation Control A 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

Samples containing EDTA cannot be used because EDTA is a strong inhibitor of ADAMTS13 function.

ADAMTS13 results on Ceveron s100 are not affected by hemoglobin up to 500 mg/dL, bilirubin* up to 18 mg/dL, lipemia up to 1400 mg/dL Intralipid™, rheumatoid factor up to 600 IU/mL and VWF up to 6 IU/mL

*In case of clear visual presence of icterus in the plasma sample, a pre-dilution of the sample 1:5 using 0.9 % sodium chloride solution is recommended. The result from the diluted sample must be multiplied by the dilution factor (x5).

INTERPRETATION OF RESULTS

ADAMTS13 Activity results are reported in IU/mL. The results can also be converted into % where 100 % equals 1.00 IU/mL.

REFERENCE RANGE

Reference range (n = 124) for TECHNOFLUOR ADAMTS13 activity: 0.60 – 1.21 IU/mL (equivalent to 60 – 121 %)

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

PERFORMANCE CHARACTERISTICS

Performance data are given below. Results obtained in individual laboratories may differ.

Clinical Performance

A single center method comparison was performed with samples covering the whole assay range comparing TECHNOFLUOR ADAMTS13 Activity with TECHNOZYM ADAMTS13 Activity ELISA (n=127).

Method	Slope	Intercept	Correlation
ELISA	1.059	-0.005	0.972

Precision

Reproducibility was determined with different samples

Sample code	Assigned value [IU/mL]	CV % within run	CV % between run
Plasma Sample 1	0.075	10.6 %	11.6 %
Plasma Sample 2	0.154	11.7 %	11.7 %
Plasma Sample 3	0.407	5.6 %	6.0 %
Plasma Sample 4	0.775	5.6 %	6.4 %
Plasma Sample 5	1.206	3.3 %	5.3 %

Limit of quantification and assay range

Lower Limit of Detection: 0.004 IU/mL

Linear Range: 0.01 – 1.25 IU/mL

STANDARDISATION

Coagulation Reference (Calibrator) is calibrated using TECHNOFLUOR ADAMTS13 Activity and traceable to the WHO International Standard for ADAMTS13 Activity, and ranges for Coagulation Control N and Coagulation Control A are assigned via analysis with that calibrator and reagent pairing.

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

lot


Storage temperature

Determinations


Expiry date /

Catalogue number


Consult instructions for use

Global Trade Item Number


Biological risk

Reaction buffer

Research use only

Substrate

Distilled water

Dilute or disolve in


Manufacturing date

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