REF **5450601** **TECHNOZYM ADAMTS13 Antigen ELISA 96 T.** ENG

REF **5450661** **TECHNOZYM ADAMTS13 Antigen CAL Set 5 x 0.5 mL**

REF **5450663** **TECHNOZYM ADAMTS13 Antigen CONT Set 2 x 0.5 mL**

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RUO

TECHNOZYM ADAMTS13 Antigen ELISA - English

INTENDED USE

The TECHNOZYM ADAMTS13 Antigen ELISA is a quantitative test for use in research activities related to the determination of ADAMTS13 antigen in human plasma, using venous-drawn fresh and/or frozen human citrated (3.2 % sodium citrate) platelet poor plasma. The assay is performed on microplate readers capable of reading a wavelength of 450 nm. By performing ADAMTS13 antigen assay in combination with an activity and autoantibody assay it is possible to differentiate between activity neutralizing and non-neutralizing autoantibodies. The TECHNOZYM ADAMTS13 Antigen ELISA is intended for use in laboratories by professionals, qualified to perform ELISA-based assays.

The TECHNOZYM ADAMTS13 Antigen CAL Set is used for calibrating the TECHNOZYM ADAMTS13 Antigen ELISA.

The TECHNOZYM ADAMTS13 Antigen CONT Set is used for quality control of the TECHNOZYM ADAMTS13 Antigen ELISA.

SUMMARY

ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type 1 motif 13) is an enzyme (vWF-cleaving protease or vWF-CP) that specifically cleaves von Willebrand factor (vWF) multimers, which induce platelet thrombus formation under high shear stress. A functional defect of this enzyme leads to the presence of higher molecular weight forms of vWF and thus to increased platelet aggregation, mainly in the microvasculature. This is believed to be the major cause for thrombotic thrombocytopenic purpura (TTP)

REAGENTS

The TECHNOZYM ADAMTS13 Antigen ELISA contains:

	Reagent / Content	Description
12 x 8 wells	ELISA test strips	Microtiterplate coated with a monoclonal anti ADAMTS13 antibody, directed against the CUB domain; Drying agent included in aluminum bag
5 x 1 x 0.5 mL	Calibrator plasma*	Numbered from 1 to 5; lyophilized; with lot-specific concentrations (values see batch table);
2 x 1 x 0.5 mL	Control plasma**	High and low control plasma; lyophilized, with lot- specific concentrations (values see batch table)
1 x 0.3 mL	Conjugate	Anti-ADAMTS-13 POX; dyed blue; liquid, ready to use
1 x 12 mL	TMB Substrate	Tetramethylbenzidine substrate; liquid, ready to use
1 x 80 mL	Wash buffer concentrate	buffer concentrate PBS; pH 7.3; containing detergent; 0.01 % merthiolate; liquid
1 x 90 mL	Incubation Buffer / Sample dilution Buffer	PBS; pH 7.3; contains stabilizer protein; 0.05% proclin; and dye: liquid, ready to use
1 x 12 mL	Stop solution	Sulphuric acid 2.5%; liquid, ready to use
2 pcs	Plate sealer	ELISA Plate sealer during incubation process

*Additionally, the calibrators are sold separately as TECHNOZYM ADAMTS13 Antigen CAL Set 5 x 0.5 mL (REF 5450661).

**Additionally, the controls are sold separately as TECHNOZYM ADAMTS13 Antigen CONT Set 2 x 0.5 mL (REF 5450663).

Material required (not supplied with the kit)

- Distilled water
- Measuring cylinder (1000 mL)
- Precision pipettes (50, 100 and 1000 µL)
- Variable pipette (100 and 1000 µL)
- Multichannel and/or dispensing pipettes (100 and 200 µL)
- ELISA washer or multichannel pipette
- ELISA reader with 450 nm filter, with a 620 nm reference filter if available
- Laboratory timer

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.
- Calibrators and 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. 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.

Symbol	Warning and Precautions	Product
	H315 Causes skin irritation. H319 Causes serious eye irritation. P264 Wash hands thoroughly after handling. Contains sulphuric acid.	Stop solution
	H317 May cause an allergic skin reaction. P280 Wear protective gloves. Contains methylisothiazol.	Incubation buffer

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:

Material / Reagent	State	Storage	Stability
ELISA test strips	After opening	2…8 °C with adhesive film in aluminium bag with drying agent	Expiry date
Calibrators, control plasmas	After reconstitution	≤ -20 °C	6 months
Conjugate	After opening	2…8 °C	6 months
	Working solution	Room temperature (18…25 °C)	60 minutes
TMB Substrate	After opening	2…8 °C	Expiry date
Wash Buffer concentrate	After opening	2…8 °C	6 months
Washing Buffer	1+11.5 dilution of concentrate	2…8 °C	3 weeks
Incubation Buffer / Sample dilution Buffer	After opening	2…8 °C	2 Months
Stop Solution	After opening	2…8 °C	Expiry date

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.

Fresh plasma samples must be measured within three hours. At -20°C they can be stored for several months. Samples may not be frozen and thawed several times.

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

Samples are used undiluted.

Preparation of reagents

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

When reconstituting plasmas, mixing reagents or buffers avoid foaming.

- *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.
- *Conjugate working solution:* Dilute 1 part by volume washing buffer concentrate with 50 parts by volume incubation buffer (1+50). Mix well!
  Mix 10 µL conjugate with 500 µL incubation buffer for 8 test wells.
- *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. Calibrator and control plasmas are used undiluted.
  Reconstituted components are clear to slightly turbid. When freezing calibrator or control plasmas the minimum volume should be 150 µL!

Performance of the test

SAMPLE INCUBATION (reference 1,2,7)	Pipette calibrators, control plasmas, samples into test wells	50 µL
	Incubate at room temperature	120 minutes
WASHING (reference 1,3,4)	Washing buffer	4 x 250 µL
CONJUGATE REACTION (reference 1,2,7)	Pipette conjugate working solution into wells	50 µL
	Incubate at room temperature	60 minutes
WASHING (reference 1,3,4)	Washing buffer	4 x 250 µL
SUBSTRATE REACTION (reference 1,2,7)	Pipette TMB Substrate solution into test wells	50 µL
	Incubate at room temperature	15 minutes
STOPPING (reference 1,2)	Pipette stopping solution into wells	50 µL
MEASUREMENT (reference 5)	ELISA reader, 450 nm	shake 10 sec., measure within 10 min.

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, 10 sec. with Vortex Mixer.
   - Test calibrators, controls and samples in duplicates.
   - Incubate at indicated temperature (RT: room temperature, 18…25 °C).
   - 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 ± 5 %.
   - During sample incubation and conjugate reaction, the time for pipetting calibrators / control plasmas / samples 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. By measuring the difference in wave lengths at 450 and 620 nm the precision of the test is increased.
6. A calibration curve has to be created for every assay.
7. For every incubation step, the test plate has to be covered with plate sealer.

LIMITATION OF THE TEST

It cannot be excluded that certain forms of ADAMTS13 (with mutations in the CUB domains) are not equivalently measured due to reduced binding to the capture antibody on the plate.

Thrombin is reported to degrade ADAMTS13. Therefore serum samples should be avoided.

INTERPRETATION OF RESULTS

TECHNOZYM ADAMTS13 Antigen results are reported in IU/mL.

CALCULATION OF RESULTS

Setting up a reference curve:

X axis: ADAMTS13 Activity [IU/mL]

Y axis: Extinction at 450 nm

Graph plot is linear-linear with a cubic spline, linear or point to point fit.

Assessment of reference curve:

The validity of the test may be checked based on the calculated control values.

Measuring concentration of samples:

Read off the concentration from the reference curve.

If there are samples, with extinction coefficients higher than the extinction of the highest point on the calibration curve, they have to be pre diluted with incubation buffer (1+1 or 1+3). The measured concentration then has to be multiplied with the dilution factor 2 or 4, respectively.

REFERENCE RANGE

Normal range for ADAMTS13 Antigen: 0.41 – 1.41 IU/mL.

It is recommended that individual laboratories establish their own normal range. When interpreting the serological results, the history of the sample has to be taken into account.

PERFORMANCE CHARACTERISTICS

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

Precision

Reproducibility was determined with different samples.

Sample code	Assigned value [IU/mL]	CV % within run	CV % total
High control	0.55	6.36	9.97
Low control	0.11	5.71	6.62

Correlation with antigen in TECHNOZYM ADAMTS13 fluorogenic method is r^2: 0.9 for normal samples and r^2:0.91 for TTP samples.

Limit of detection and assay range

When assay is performed as indicated in section 'Test Procedure', the detection limit of this assay is 0.012 IU/mL. The upper limit of the assay range may vary with each lot of kit depending on the assayed value of the calibrator plasma supplied in the kit. Samples with values outside the range of the reference curve should be re-tested at an appropriate dilution to obtain accurate results.

STANDARDISATION

Standards and controls are produced from a normal donor and they are calibrated against the WHO International Standard for ADAMTS13. Consult the batch table.

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

RUO Research use only

 Storage temperature

LOT lot

 Expiry date

REF Catalogue

 Consult instructions for use

GTIN Global Trade Item Number

 Biological

BUF Reaction buffer

WASH Washing solution concentrate

CAL Calibrator

INC Incubation buffer

CONT Control

SUB substrate

CONJ Conjugate

STOP stop solution

MTP Microtiter plate

RTU Ready to use

 Determinations

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