ENG

REF **5450501** **TECHNOZYM ADAMTS13 Activity/Antigen ELISA 2 x 48 T.**

REF **5450551** **TECHNOZYM ADAMTS13 Activity/Antigen ELISA 48 T.**

REF **5450561** **TECHNOZYM ADAMTS13 Activity/Antigen CAL Set 5 x 0.5 mL**

REF **5450563** **TECHNOZYM ADAMTS13 Activity/Antigen CONT Set 2 x 0.5 mL**

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TECHNOZYM ADAMTS13 Activity/Antigen ELISA - English

INTENDED USE

The TECHNOZYM ADAMTS13 Activity/Antigen ELISA is a quantitative fluorogenic test for the determination of ADAMTS13 activity and 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 flourescence microplate readers with suitable wavelength ranges. 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 Activity/Antigen ELISA is intended for prescription use in laboratories by professionals, qualified to perform ELISA-based assays.

The TECHNOZYM ADAMTS13 Activity/Antigen CAL set is used for calibrating the TECHNOZYM ADAMTS13 Activity/Antigen ELISA.

The TECHNOZYM ADAMTS13 Activity/Antigen CONT Set is used for quality control of the TECHNOZYM ADAMTS13 Activity/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 unusually large von Willebrand factor (vWF) multimers, which induce platelet thrombus formation under high shear stress. If the activity of ADAMTS13 is lowered for some reason, however, unusually large vWF multimers may accumulate within blood, causing thrombosis due to platelet aggregation, which in turn may lead to TMA (thrombotic microangiopathy) such as TTP (thrombotic thrombocytopenic purpura).

REAGENTS

The TECHNOZYM ADAMTS13 Activity/Antigen kit contains:

	Reagent / Content	Description
6 x 8 wells for 48T. 12 x 8 wells for 2x48T.	ELISA test stripes	Microtiterplate coated with a monoclonal anti-ADAMTS13 antibody, directed against the CUB domain; the drying agent is supplied in an aluminium bag
1 x 80 mL	Washing buffer concentrate	PBS; pH 7.3; containing detergent; 0.01% merthiolate; liquid
1 x 90 mL	Incubation buffer	PBS; pH 7.3; contains stabiliser protein; 0.05 % proclin; and dye, 1 vial, 90 mL; liquid, ready to use.
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 3 mL for 48T. 2 x 3 mL for 2 x 48T.	Activity substrate	lyophilized
1 x 0.3 mL	Conjugate	anti-ADAMTS13 POX; dyed blue; liquid
1 x 6 mL	Antigen substrate	Tetramethylbenzidine Substrate; liquid
1 x 0.7 mL	Stable peroxyde solution	Liquid
1 x 6 mL	Stop solution for antigen substrate	Sulphuric acid 0.5 M; liquid, ready to use
2 pcs	Plate sealer	ELISA Plate sealer used during incubation process

*Additionally, the calibrators are sold separately as TECHNOZYM ADAMTS13 Activity/Antigen CAL Set (REF 5450561).

**Additionally, the controls are sold separately as TECHNOZYM ADAMTS13 Activity/Antigen CONT Set (REF 5450563).

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
- Fluorescence reader, with suitable wavelength ranges, see references11, 12. Please note that monochromators and some fluorescence reader brands are not recommend for this assay. The list of available applications can be found under www.technoclone.com.
- 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 HbsAg, HIV 1/2 Ab and HCV-Ab-negative as tested by FDA approved 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.

Symbol	Warning and Precausion	Product
	H315 causes skin irriation P264 wash hands thoroughly after handling Contains sulphuric acid	Stop solution

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
Calibrators, control plasmas	after reconstitution	≤ -20 °C	6 months
ELISA test strips	after opening	2…8 °C with adhesive film in aluminium bag with drying agent	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	after opening	2...8 °C	2 months
Activity substrate	After reconstitution	≤ -20 °C	2 months
Conjugate	after opening	2...8 °C	6 months
	working solution	room temperature (18…25 °C)	60 minutes
Antigen substrate	after opening	2...8 °C	Expiry date
Antigen substrate working solution	9+1 mixture of substrate and stable peroxide solution	room temperature (18…25 °C)	24 hours
Stable peroxide solution	after opening	2...8 °C	Expiry date
Stop solution for antigen substrate	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. Samples may be stored for three hours at room temperature.

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.

Please note: maximum 6 strips (48T.) can be measured per assay.

- *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.
- *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. Calibrators and control plasmas are used undiluted. Reconstituted components are clear to slightly turbid. When freezing calibrators or control plasmas the minimum volume should be 150 µL!
- *Activity substrate:* Activity substrate is reconstituted with 3 mL distilled water and mixed gently for 10 seconds after a reconstitution time of 15 minutes stored in the dark.
  The activity substrate has to be used immediately after end of reconstitution time.
- *Conjugate working solution (1+50):* Dilute 1 part by volume conjugate with 50 parts by volume incubation buffer.
  Mix 10 µL conjugate with 500 µL incubation buffer for 8 test wells.
- *Antigen substrate working solution (9+1):* Mix 9 parts antigen substrate with 1 part stable peroxide solution.
  Mix 450 µL antigen substrate with 50 µL stable peroxide solution for 8 test wells.

Performance of the test

SAMPLE INCUBATION (reference 1, 2, 3, 5, 10, 13, 15)	Pipette calibrators, control plasmas, samples into test wells	50 µL
	Incubate at room temperature	120 minutes (2 hours)
WASHING (reference 1, 3, 4)	Washing buffer	3 x 200 µL
ACTIVITY MEASUREMENT (reference 1, 2, 6, 8, 9, 11)	Activity substrate	50 µL
	Measure kinetic at 30 °C; 340/450 nm;	15 minutes (1 measurement per minute)
WASHING (reference 1, 3, 4)	Washing buffer	3 x 200 µL
CONJUGATE REACTION (reference 1, 2, 15)	Pipette conjugate working solution into wells	50 µL
	Incubate at room temperature	60 minutes
WASHING (reference 1, 3, 4)	Washing buffer	3 x 200 µL
ANTIGEN-SUBSTRATE REACTION (reference 1, 2, 7, 15)	Pipette antigen substrate working solution into test wells	50 µL
	Incubate at 30 °C or 37 °C	15 minutes
STOPPING (reference 1, 2)	Pipette stopping solution into wells	50 µL
ANTIGEN MEASUREMENT (reference 12)	Read end point, 325/410 nm;	shake 10 sec., measure within 5 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.
   - Run calibrators, controls and samples in duplicates.
   - Incubate at indicated temperature (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 reactions 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 pipettes.
3. Label / number strips with a water-resistant white 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. The last remnants must be removed by gentle tapping.
5. Maximum 6 strips can be measured in one assay.
6. For BioTek FLx800 sensitivity for Activity measurement has to be set to 130-150 so that measured value of Calibrator 1 is in a range of ~300-700 RFU/min.
7. For BioTek FLx800 sensitivity for Antigen measurement has to be set to 40-60 so that measured value of Calibrator 1 is in a range of ~300-700 RFU/min
8. For antigen determination substrate incubation is possible at 30 °C or 37 °C.
9. The kinetic reading has to be started immediately after pipetting the activity substrate to the plate.
10. For using the TECHNOZYM ADAMTS13 evaluation software the plate layout has to be chosen as indicated: Standards have to be measured in duplicate, the duplicates placed in columns, one below the other. Controls and samples can be measured as single values but it is recommended to do them in duplicate as well. If done as duplicate, they also have to be placed one below the other. Plate layout has to be followed strictly as evaluation program can't work properly otherwise.
11. Activity measurement: suitable wavelength range for excitation 320 - 360 nm and for emission 440 – 460 nm. For BioTek reader FLx800 TBI: 360/460 nm for excitation/emission.
12. Antigen measurement: suitable wavelength range for excitation 315 - 340 nm and for emission 370 – 470 nm. For BioTek reader FLx800 TBI: 360/460 nm for excitation/emission.
13. A calibration curve has to be created for every assay.
14. No agitation is required during each reaction step.
15. For every incubation step, the test plate has to be covered with plate sealer.

LIMITATION OF THE TEST

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

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

It can not 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.

Please note that monochromators and some fluorescence reader brands are not recommend for this assay. The list of available applications can be found at www.technoclone.com.

INTERPRETATION OF RESULTS

TECHNOZYM ADAMTS13 Activity/Antigen results are reported in IU/mL ADAMTS13 activity and IU/mL ADAMTS13 antigen..

CALCULATION OF RESULTS

ADAMTS13 Activity Determination

Setting up a reference curve:

X axis: ADAMTS13 Activity [IU/mL]

Y axis: RFU/min (slope of kinetic curve)

Graph plot is linear-linear with a best fit.

Assessment of reference curve:

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

Coefficients of variation of duplicates shouldn't exceed 15%.

Measuring concentration of samples:

Read off the concentration from the reference curve.

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

ADAMTS13 Antigen Determination

Setting up a reference curve:

X axis: concentration ADAMTS13 Antigen [IU/mL]

Y axis: relative fluorescence units (RFU)

Graph plot is linear-linear with a best fit.

Assessment of reference curve:

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

Coefficients of variation of duplicates shouldn't exceed 15%.

Measuring concentration of samples:

Read off the concentration from the reference curve.

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

Evaluation software

The software "TECHNOZYM ADAMTS13 evaluation software" for both, Activity and Antigen determination will be provided on request (sales@technoclone.com) or as download at www.technoclone.com.

It is recommended to use this evaluation software in combination with the respective reader software.

REFERENCE RANGE

Normal range for ADAMTS13 Activity: 0.31-1.31 IU/mL

Normal range for ADAMTS13 Antigen concentration: 0.37-1.33 IU/mL

Normal range can vary depending on local population. 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	
	Activity	Antigen	Activity	Antigen	Activity	Antigen
High control	0.83	0.86	5.83	4.26	1.71	1.66
Low control	0.09	0.12	7.44	7.79	7.94	4.93

Limit of quantification and assay range

When assay is performed as indicated in section 'Test Procedure', the limit of quantification of this assay is 0.02 IU/mL activity and 0.02 IU/mL antigen. 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.

EDITORAL 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

RUO Research use only

LOT lot

REF Catalogue

GTIN Global Trade Item Number

BUF Reaction buffer

WASH Washing solution concentrate

INC Incubation buffer

SUB substrate

STOP stop solution

RTU Ready to use

CAL Calibrator

CONT Control

CONJ Conjugate

MTP Microtiter plate

Σ Determinations

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