REF **5450701** **TECHNOZYM ADAMTS13 Activity ELISA 96 T.** ENG

REF **5450761** **TECHNOZYM ADAMTS13 Activity CAL Set 6 x 0.5 mL**

REF **5450763** **TECHNOZYM ADAMTS13 Activity CONT Set 2 x 0.5 mL**

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TECHNOZYM ADAMTS13 Activity ELISA - English

INTENDED USE

The TECHNOZYM ADAMTS13 Activity ELISA is a chromogenic, semi-quantitative test for use in research activities related to the measurement of ADAMTS13. The assay requires venous-drawn fresh and/or frozen human citrated (3.2 % sodium citrate) platelet poor plasma and it is performed withmicroplate readers capable of reading a wavelength of 450 nm.

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

The TECHNOZYM ADAMTS13 Activity CONT Set is used for quality control of the TECHNOZYM ADAMTS13 Activity 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), 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, 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 ELISA contains:

	Reagent / Content	Description
12 x 8 wells	ELISA test stripes	Microtiterplate coated with a monoclonal anti GST-Antibody; Drying agent included in aluminum bag
2 x 6 mL	GST-vWF73 Substrate	lyophilized
6 x 1 x 0.5 mL	Calibrator plasma*	Numbered from 1 to 6; 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 30 mL	Reaction buffer	Liquid, ready to use
1 x 12 mL	Conjugate	HRP conjugated monoclonal Antibody; liquid, ready to use
1 x 12 mL	TMB Substrate	Tetramethylbenzidine substrate; liquid, ready to use
1 x 53 mL	Wash buffer concentrate	10-fold concentrated
1 x 12 mL	Stop solution	Sulphuric acid 2.5 %; liquid, ready to use
1 x 1 plate	Sample dilution microplate	Only for Sample dilution! (Not coated)
3 pcs	Plate sealer	ELISA Plate sealer during incubation process

*Additionally, the calibrators are sold separately as TECHNOZYM ADAMTS13 Activity CAL Set 6 x 0.5 mL (REF 5450761).

**Additionally, the controls are sold separately as TECHNOZYM ADAMTS13 Activity CONT Set 2 x 0.5 mL (REF 5450763)

Material required (not supplied with the kit)

- Distilled water
- Measuring cylinder (500 mL)
- Precision pipettes (5, 50, 100 and 1000 µL)
- Variable pipette (200 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

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 aluminum bag with drying agent	6 weeks
GST-vWF73 Substrate	After reconstitution	≤ -20 °C	6 weeks
Calibrators, control plasmas	After reconstitution	≤ -20 °C	6 weeks
Reaction Buffer	After opening	2...8 °C	6 weeks
Conjugate	After opening	2...8 °C	6 weeks
TMB Substrate	After opening	2...8 °C	6 weeks
Wash Buffer (10-fold concentrate)	After opening	2...8 °C	6 weeks
Washing Buffer	1+9 dilution of concentrate	2...8 °C	6 weeks
Stop Solution	after opening	2...8 °C	6 weeks

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 if necessary. Gently mix before testing. After thawing, the assay must be performed within 2 hours. Samples may be frozen once at -20 °C. When freezing samples the minimum volume should be 150 µL!

- *Sample dilution:* Dilute samples, 31-fold with reaction buffer in a sample dilution Microplate.

  Example: 150 µL reaction buffer + 5 µL sample
  For higher precision, volumes can be up scaled, using larger tubes for dilution:
  e.g. 600 µL reaction buffer + 20 µL sample

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 9 parts by volume distilled water (1+9). Mix well! (diluted washing buffer concentrate = washing buffer). There may be crystalline precipitations which will dissolve at 37 °C within 10 minutes.
- *GST-vWF73 Substrate solution:* Substrate solution is reconstituted with 6 mL distilled water and mixed for 10 seconds after a reconstitution time of 15 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. Dilute calibrators and controls 31-fold with reaction buffer in a sample dilution Microplate. (See sample dilution).
  Reconstituted components are clear to slightly turbid.
  When freezing calibrator or control plasmas the minimum volume should be 150 µL!

Performance of the test

GST-vWF73 SUBSTRATE INCUBATION (reference 1,2,3,7,9)	Add GST-vWF73 Substrate solution to anti-GST coated test strips	100 µL
	Incubate at room temperature	60 minutes
WASHING (reference 1,3,4)	Washing buffer	3 x 300 µL
SAMPLE INCUBATION (reference 1,2, 5,6,7,9,10)	Pipette diluted calibrators, control plasmas, samples into test wells	100 µL
	Incubate at room temperature	30 minutes
WASHING (reference 1,3,4)	Washing buffer	3 x 300 µL
CONJUGATE REACTION (reference 1,2,7,9)	Pipette conjugate into wells	100 µL
	Incubate at room temperature	60 minutes
WASHING (reference 1,3,4)	Washing buffer	3 x 300 µL
TMB COLOR REAGENT REACTION (reference 1,2,7,9)	Pipette TMB substrate into test wells	100 µL
	Incubate at room temperature	30 minutes
STOPPING (reference 1,2,7)	Pipette stopping solution into wells	100 µL
MEASUREMENT (reference 8)	ELISA reader, 450 nm	shake 5 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.
   - 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 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. A calibration curve has to be created for every assay.
6. Samples / calibrators / controls can be transferred from sample dilution microplate to anti-GST microplate by multichannel pipette. Do not forget to change tips for every strip!
7. No agitation is required during each reaction step.
8. By measuring the difference in wavelength at 450 nm and 620 nm, the precision of the test is increased.
9. For every incubation step, the test plate has to be covered with plate sealer.
10. It is not mandatory to use all six calibrators for creating a calibration curve. For sample screening, a calibration curve using calibrators 1, 2, 3, 4, and 6 is sufficient. When the focus is particularly in the lower range of ADAMTS13 activity, a calibration curve with calibrators 2, 3, 4, 5 and 6 is adequate.

LIMITATION OF THE TEST

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

Hemolysis: No interference is observed with samples containing up to 200 mg/dL hemoglobin, which corresponds with a moderate hemolysis.

Lipemia: No interference is observed with samples containing up to 300 mg/dL Intralipid™, which corresponds with a moderate to severe concentration.

Icterus: No interference was observed with samples containing up to 15 mg/dL bilirubin (conjugated as well as unconjugated), which corresponds with a moderate to severe concentration.

Rheumatoid factor: No interference was observed up to 28 IU/mL, with corresponds with a 2-fold concentration of normal.

Anti CD20 antibodies: No interference was observed up to a level of 200 µg/mL, which corresponds to the upper level of serum concentrations found after Rituximab administration.

INTERPRETATION OF RESULTS

TECHNOZYM ADAMTS13 Activity 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 best 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 reaction 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 Activity: 0.4 – 1.3 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.

Performance

Performance was determined in samples with TTP.

Parameter	Value	95 % CI
Sensitivity	98.73 %	93.15 % to 99.97 %
Specificity	92.86 %	80.52 % to 98.50 %
Positive Predictive Value	96.30 %	89.73 % to 98.72 %
Negative Predictive Value	97.50 %	84.74 % to 99.64 %

Assay performance parameters were calculated based on a 0.1 IU/mL cut off.

Precision

Reproducibility was determined with different samples.

Sample code	Assigned value [IU/mL]	CV % within run	CV % total
High control	0.909	7.7	10.5
Low control	0.239	9.3	13.8

Limit of quantification and assay range

When assay is performed as indicated in section ‘Test Procedure’, the limit of quantification of this assay is 0.0071 IU/mL ADAMTS13 activity. 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

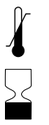
Please contact Technoclone www.technoclone.com or your local distributor.

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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