

REF 5450701-RUO TECHNOZYM[®] ADAMTS-13 Activity ELISA
REF 5450761-RUO TECHNOZYM[®] ADAMTS-13 Activity Calibration Set
REF 5450763-RUO TECHNOZYM[®] ADAMTS-13 Activity Control Set

3050192RUO Rev. 018 [01] 08/2018

For research use only

TECHNOZYM[®] ADAMTS-13 Activity ELISA - English

INTENDED USE

The TECHNOZYM[®] ADAMTS-13 Activity ELISA is a quantitative test for aid in the research of TTP, using venous-drawn fresh and/or frozen human citrated (3.2% sodium citrate) platelet poor plasma, only when considered in conjunction with other clinical indicators. The assay is performed on microplate readers capable of reading a wavelength of 450 nm. The TECHNOZYM[®] ADAMTS-13 Activity ELISA is a chromogenic test for measurement of ADAMTS-13 activity. ADAMTS-13 activity measurement serves as an aid in the research of TTP. The TECHNOZYM[®] ADAMTS-13 Activity ELISA is intended for prescription use in laboratories by professionals qualified to perform ELISA-based assays.

The TECHNOZYM[®] ADAMTS-13 Activity Calibration Set is used for calibrating the TECHNOZYM[®] ADAMTS-13 Activity ELISA.

The TECHNOZYM[®] ADAMTS-13 Activity Control Set is used for quality control of the TECHNOZYM[®] ADAMTS-13 Activity ELISA.

SUMMARY

ADAMTS-13 (a disintegrin-like and metalloproteinase with thrombospondin type 1 motif 13) is an enzyme (wWF-cleaving protease or wWF-CP) that specifically cleaves unusually large von Willebrand factor (wWF) multimers, which induce platelet thrombus formation under high shear stress. If the activity of ADAMTS13 is lowered for some reason, however, unusually large wWF 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

- ELISA test strips (12), with 8 wells each, coated with a monoclonal anti GST- Antibody. The drying agent is supplied in an aluminum bag.
- GST-wWF73 Substrate; 2 vials; lyophilized; 6 mL.
- Calibrators numbered from 1 to 6; lyophilized; 1 vial each; 0.5 mL. Concentrations are lot-specific; consult the batch table.
- Additionally, the calibrators are sold separately as TECHNOZYM[®] ADAMTS-13 Activity Calibration Set (REF 5450761)
- High and low control plasma, lyophilized, 1 vial each, 0.5 mL. Concentrations are lot-specific; consult the batch table.
- Additionally, the controls are sold separately as TECHNOZYM[®] ADAMTS-13 Activity Control Set (REF 5450763)
- Reaction buffer; 1 vial; 30mL, ready to use.
- Conjugate: HRP conjugated monoclonal Antibody; 1 vial; 12 mL; ready to use.
- TMB Substrate (Tetramethylbenzidine); 1 vial; 12mL; ready to use.
- Wash buffer concentrate, 10-fold concentrated, 1 vial, 53 mL.
- Stop solution; sulphuric acid 0.5 M, 1 vial, 12 mL; ready to use.
- Sample dilution Microplate, 1 plate (ONLY for sample dilution!).
- Plate sealer 3 pieces.

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

- 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.
- 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 Precaution	Product
	H315 causes skin irritation P264 wash hands thoroughly after handling Contains sulphuric acid	Stop solution

Stability and storage

The expiry date printed on the labels applies to storage of the unopened vial at + 2...8 °C. Stability after reconstitution/opening:

Material/ Reagent	State	Storage	Stability
ELISA test strip	after opening	2...8 °C with adhesive film in plastic bag with drying agent	6 weeks
GST-wWF73 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
Wash Buffer	1+9 dilution of concentrate	2...8 °C	6 weeks
Stop solution	after opening	2...8 °C	6 weeks

TEST PROCEDURE

Preparation of the samples

Sample material: citrated human plasma. Blood is collected into 3.2 % Buffered Citrate anticoagulant tubes at a ratio of 9 volumes blood to 1 volume anticoagulant and gently mixed by inversion. Centrifuge at a minimum of 1500 x g for 15 minutes (CLSI Guideline H21-A5) and remove supernatant plasma. Samples may be stored up to two hours at room temperature. Samples may be frozen once at ≤ -20°C.

Preparation of reagent

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

Preparing the 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.

Reconstituting GST-wWF73 Substrate Solution: Substrate Solution is reconstituted with 6mL distilled water and mixed for 10 seconds after a reconstitution time of 15 minutes (vortex mixer).

Reconstituting 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 (vortex mixer).

Reconstituted components are clear to slightly turbid.

Sample / Calibrator / Control dilution: dilute samples, calibrators and controls 31-fold with reaction buffer in a sample dilution Microplate

Example: 150 µL Reaction buffer + 5 µL Sample / Calibrator / Control

For higher precision, volumes can be upscaled, using larger tubes for dilution:

e.g. 600 µL reaction buffer + 20 µL Sample / Calibrator / Control

Performance of the test

GST-wWF73 SUBSTRATE INCUBATION (reference 1,2,3,7,9)	Add GST-wWF73 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 300µL
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 300µL
TMB COLOUR 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.
 - Test 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; by gentle tapping, the last remnants must be removed.
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 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 ADAMTS-13 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 ADAMTS-13 function.

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

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 RF, 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.

CALCULATION OF RESULTS

Setting up a reference curve:

X axis: ADAMTS-13 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 that of the highest point on the 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.

REFERENCE RANGE

Normal range for ADAMTS-13 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.

Standardisation

Standards and controls are produced from a normal donor and they are calibrated against the WHO International Standard for ADAMTS-13.

PERFORMANCE CHARACTERISTICS

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

Clinical Performance

Performance was determined in samples with proven and suspected 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 %

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 ADAMTS-13 activity. The upper limit of detection 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.

LITERATURE

Please contact Technoclone or your local distributor.

SYMBOLS KEY

	Manufacturer	RTU	Ready to use
	Storage temperature	CAL	Calibrator
	Expiry date	CONT	Control
	Consult instructions for use	CONJ	Conjugate
	Determinations	BUF	Reaction Buffer
GTIN	Global Trade Item Number	SUB	Substrate
	Biological risk	STOP	Stop solution
RUO	For research use only	WASH	Washing solution concentrate
LOT	Lot	MTP	Microtiter plate
REF	Catalogue number		