Technozym ADAMTS13 Activity

Intended Use

The Technozym ADAMTS13 Activity assay is an enzyme-linked immunosorbent assay (ELISA) intended for the qualitative determination of ADAMTS13 activity in platelet poor human citrated plasma. The assay is intended to be used in conjunction with other clinical and laboratory findings as an aid in the diagnosis of thrombotic thrombocytopenic purpura (TTP) in adult and pediatric patients being evaluated for thrombotic microangiopathy (TMA).

Summary and principle

ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type 1 motif, 13) is an enzyme that specifically cleaves von Willebrand factor (VWF) multimers which induce platelet thrombus formation under high shear stress. If the activity of ADAMTS13 is decreased either due to the presence of autoantibodies or because of a mutation within the ADAMTS13 gene, unusually large VWF multimers may accumulate, causing microvascular thrombosis due to platelet aggregation, which in turn may lead to thrombotic thrombocytopenic purpura (TTP), a potentially fatal thrombotic microangiopathy.

The Technozym ADAMTS13 Activity assay is an ELISA (Enzyme Linked Immuno Sorbent Assay) for the detection of ADAMTS13 activity in human citrated plasma. Polystyrene microtiter wells are pre-coated with an antibody specific to gluthathione S transferase (GST). In a first step, GST-VWF73 is added to each well, whereupon the GST part of the tagged VWF73 binds to the anti-GST antibody. After a washing step, calibrators, controls and samples, diluted in reaction buffer, are added to the plate in appropriate locations and incubated. The ADAMTS13 present in the samples specifically cleaves the VWF73 Substrate bound to the plate, exposing the neoepitope of the cleavage site. After washing, a ready-to use conjugate solution comprising a horseradish peroxidase (HRP) - labeled monoclonal antibody specific to the cleavage site is added to each well and incubated. After washing, wells are incubated with tetramethylbenzidene (TMB), an HRP substrate, which produces a colored product. An acidic stop solution is then added and the degree of enzymatic turnover – which is proportional to ADAMTS13 activity in the sample - is determined by the absorbance (optical density) measurement at 450 nm using a standard microplate reader.

The Technozym ADAMTS13 Activity assay comprises six calibrators to create a calibration curve where controls and samples are read from.

Reagents

REF	CONTENT
5450701-US	12x8 wells

Each Technozym ADAMTS13 Activity consists of:

TEST	**ADAMTS13 Activity Anti-GST Coated Test Plate**: 12 x 8 wells coated with an anti-GST antibody; drying agent included.
VWF	**ADAMTS13 Activity GST-VWF73:** 2 x 6 mL vials of lyophilized reagent containing buffer and preservatives.
CAL 1	**ADAMTS13 Activity Calibrator 1**: 1 x 0.5 mL vial of a lyophilized human plasma containing buffer and stabilizers.
CAL 2	**ADAMTS13 Activity Calibrator 2:** 1 x 0.5 mL vial of a lyophilized human plasma containing buffer and stabilizers.
CAL 3	**ADAMTS13 Activity Calibrator 3**: 1 x 0.5 mL vial of a lyophilized human plasma containing buffer and stabilizers.
CAL 4	**ADAMTS13 Activity Calibrator 4:** 1 x 0.5 mL vial of a lyophilized human plasma containing buffer and stabilizers.
CAL 5	**ADAMTS13 Activity Calibrator 5:** 1 x 0.5 mL vial of a lyophilized human plasma containing buffer and stabilizers.
CAL 6	**ADAMTS13 Activity Calibrator 6**: 1 x 0.5 mL vial of an immunodepleted lyophilized human plasma containing buffer and stabilizers.
CON H	**ADAMTS13 Activity Control High:** 1 x 0.5 mL vial of a lyophilized human plasma containing buffer and stabilizers.
CON L	**ADAMTS13 Activity Control Low:** 1 x 0.5 mL vial of a lyophilized human plasma containing buffer and stabilizers.
BUF	**ADAMTS13 Activity Reaction Buffer:** 1 x 30 mL vial of a solution containing buffer and preservatives.
CONJ	**ADAMTS13 Activity Conjugate:** 1 x 12 mL vial of HRP conjugated monoclonal antibody, buffer and preservatives.
TMB	**ADAMTS13 Activity TMB Substrate:** 1 x 12 mL vial of Tetramethylbenzidine substrate.
WASH	**ADAMTS13 Activity Wash Buffer Concentrate:** 1 x 53 mL vial of 10-fold concentrated solution containing buffer and preservatives.
STOP	**ADAMTS13 Activity Stop Solution:** 1 x 12 mL vial of sulfuric acid 2.5%.
-	**Sample Dilution Plate:** 12 x 8 wells, uncoated plate, for sample dilution ONLY!
-	**Plate sealer:** 3 pieces, for ELISA plate sealing during incubation process.

Reagents of different kit lots must not be combined. Calibrators and controls have lot-specific concentrations and cannot be used with other lots of reagents. For values see batch table.

Precautions and warnings

This product is for in vitro diagnostic use.

Rx only. Caution: Federal law restricts this device to sale by or on the order of a physician.

This kit is intended for use by personnel trained in laboratory procedures.

Universal precautions for the use of chemicals and potentially biohazardous substances must be applied.

Handle waste as potentially biohazardous material and dispose according to accepted laboratory instructions and procedures.

Discard all material in a safe and acceptable manner in compliance with relevant local disposal regulations.

The ADAMTS13 Activity anti-GST coated test plate, the ADAMTS13 Activity GST-VWF73, the ADAMTS13 Activity reaction buffer, and the ADAMTS13 Activity conjugate contain animal source material, and therefore should be treated as potentially infectious.

All human source material in this product was tested by FDA approved methods and found nonreactive for Hepatitis B surface Antigen (HBsAg), Anti-HCV and HIV 1/2 antibodies. These products, like all human based specimens, must be considered as potentially infectious and should 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.

Limitation:

In line with the recommendation of the International Society of Thrombosis and Haemostasis (ISTH)[1], a test result of >0.1 IU/mL in patients with a high clinical suspicion of TTP, should not exclude the diagnosis of TTP.

The use of the assay to guide treatment decision for plasma exchange, rituximab and caplacizumab has not been evaluated.

The assay is not evaluated for monitoring TTP relapse or recurrence.

Hazardous component:

Hazardous components classification in accordance with 29 CFR 1910 (OSHA HCS):

Hazard class	None
Hazard Statement	None
Precautionary Statement	None

Reagent handling

NOTE: All required components are to be brought to room temperature before starting the test.

Do not use the reagent if you observe any change in appearance of components included in the kit or if you observe any damage in the packaging materials.

When reconstituting plasmas, mixing reagents or buffers, foaming should be avoided.

Precision pipettes, measuring cylinder and vortex mixer are required.

Wash Buffer: Dilute 1 part by volume washing buffer concentrate with 9 parts by volume deionized water (1+9). Mix well. (Diluted washing buffer concentrate = washing buffer). There may be crystalline precipitates, which will dissolve at 37 °C within 10 minutes.

GST-VWF73: GST-VWF73 is reconstituted with 6.0 mL deionized water. After a reconstitution time of 15 minutes, GST-VWF73 is mixed for 10 seconds.

Calibrators 1-6, **Control High and Low**: Dissolve the contents of each vial with 500 µL deionized water. After a reconstitution time of 15 minutes, calibrators and controls are mixed for 10 seconds. Reconstituted components are clear to slightly turbid. Freezing calibrator or control plasma is not recommended. When freezing, calibrators or controls should be kept in their original vial at <-20 °C for up to 6 weeks. The minimum volume for freezing should be 150 µL. Samples should be frozen only once.

Calibrators and controls are diluted 31-fold with reaction buffer in a sample dilution microplate (e.g., 150 µL reaction buffer + 5 µL calibrator / control).

Anti-GST coated test plate, **Reaction Buffer, Conjugate, TMB Substrate** and **Stop Solution** are ready to use and do not need further preparation.

Reagent storage and stability

Store at 2-8 °C.

Unopened reagents, calibrators and controls are stable until the expiration date shown on the labels when stored at 2-8 °C.

ADAMTS13 Activity Anti-GST Coated Test Plate: Stability after opening at 2-8 °C with adhesive film in aluminum bag with drying agent: 6 weeks.

ADAMTS13 Activity GST-VWF73: Stability after reconstitution at ≤ -20 °C: 6 weeks.

ADAMTS13 Activity Calibrator 1 - 6: Stability after reconstitution at ≤ -20 °C: 6 weeks.

ADAMTS13 Activity Control Plasma High and Low: Stability after reconstitution at ≤ -20 °C: 6 weeks.

ADAMTS13 Activity Reaction Buffer: Stability after opening at 2-8 °C: 6 weeks.

ADAMTS13 Activity Conjugate: Stability after opening at 2-8 °C: 6 weeks.

ADAMTS13 Activity TMB Substrate: Stability after opening at 2-8 °C: 6 weeks.

ADAMTS13 Activity Wash Buffer: Stability of wash buffer concentrate after opening at 2-8 °C: 6 weeks, stability of 1+9 dilution of concentrate after preparation at 2-8 °C: 6 weeks.

ADAMTS13 Activity Stop Solution: Stability after opening at 2-8 °C: 6 weeks.

Considering the numerous possible combinations of storage conditions, each laboratory should establish its own stability durations according to its practices. These durations should not exceed the figures mentioned above which have been determined under controlled conditions.

Specimen collection and preparation

For 3.2 % citrated human plasma only.

Plasma: Nine parts of freshly drawn venous blood are collected into one part 3.2 % trisodium citrate. Refer to CLSI Document H21 (current edition)[2] for further instructions on specimen collection, handling, and storage.

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 upscaled, using larger tubes for dilution: e.g., 600 µL reaction buffer + 20 µL sample.

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

Undiluted samples may be kept at room temperature (18 – 25 °C) for up to 8 hours or refrigerated (2 – 8 °C) for up to 24 hours.

Samples may be frozen undiluted once for up to 12 months at <-20 °C. Thaw frozen samples rapidly at 37 °C using a water bath and centrifuge if necessary. Gently mix before testing. After thawing, the assay must be performed within two hours. When freezing samples the minimum volume should be 150 µL.

Procedure

Materials provided

See "Reagents" section.

Additional materials required (not supplied with the kit)

Deionized water, measuring cylinder (500 mL), precision pipettes (5 and 100 µL), variable pipettes (200 and 1000 µL), multichannel and/or dispensing pipettes (100, 150 and 300 µL), vortex mixer, ELISA washer or multichannel pipette, laboratory timer and microplate reader capable of reading a wavelength of 450 nm are required.

Test procedure

GST-VWF73 Incubation	Add **GST-VWF73** to **Anti-GST Coated Test Plate**	100 µL
	Incubate at room temperature	60 minutes
Washing	**Washing Buffer**	3 x 300 µL
Sample Incubation	Pipette 31-fold diluted **calibrators, control plasmas, samples** into test wells	100 µL
	Incubate at room temperature	30 minutes
Washing	**Washing Buffer**	3 x 300 µL
Conjugate reaction	Pipette **Conjugate** into wells	100 µL
	Incubate at room temperature	60 minutes
Washing	**Washing Buffer**	3 x 300 µL
TMB substrate reaction	Pipette **TMB Substrate** into test wells	100 µL
	Incubate at room temperature	30 minutes
Stopping	Pipette **Stop Solution** into test wells	100 µL
Measurement	Microplate reader, 450 nm	Shake 5 sec, measure within 10 min

NOTE: Precision and Performance, among others, essentially depend on the following factors:

- Thorough mixing of all substances used
- A calibration curve has to be created for every test run.
- High and low control have to be analyzed with each run.
- Calibrators, controls and samples need to be tested 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.
- Strips should be labeled / numbered with a water resistant pen, in case the strips accidentally fall out of the frame during testing.
- After each washing step, wells must be aspirated thoroughly, turned upside down and positioned on a blotting paper. The last remnants must be removed by gentle tapping.
- No agitation is required during each incubation step.
- For every incubation step, the test plate has to be covered with a plate sealer.
- The time for pipetting calibrators, control plasmas or samples must not exceed 60 seconds per ELISA test strip (8 wells). To reduce pipetting times, calibrators, controls and samples can be transferred from sample dilution microplate to anti-GST Coated Test Plate by using a multichannel pipette. Do not forget to change tips for every strip.
- The time for pipetting GST-VWF73, Conjugate, TMB Substrate or Stop Solution in the respective reaction steps must not exceed 10 seconds per microtiterplate test strip. Short pipetting times may be secured by using multichannel pipettes.
- 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 %.

Recommended Plate layout: each sample should be tested in duplicates in two consecutive wells.

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL 1		sample 1		sample 9		sample 17		sample 25		sample 33	
B	CAL 2		sample 2		sample 10		sample 18		sample 26		sample 34	
C	CAL 3		sample 3		sample 11		sample 19		sample 27		sample 35	
D	CAL 4		sample 4		sample 12		sample 20		sample 28		sample 36	
E	CAL 5		sample 5		sample 13		sample 21		sample 29		sample 37	
F	CAL 6		sample 6		sample 14		sample 22		sample 30		sample 38	
G	CON H		sample 7		sample 15		sample 23		sample 31		sample 39	
H	CON L		sample 8		sample 16		sample 24		sample 32		sample 40	

Traceability of Calibrators and Control Materials

The reported values were determined over multiple runs using multiple reagent lots and against an internal House Standard, which has been value assigned against the current International Reference material for ADAMTS13 (WHO 1st international Standard ADAMTS13, plasma 12/252)[3].

Quality Control

A high and low control need to be analyzed in duplicates within each test run. When the control values fall within range the assay is valid and patient results can be evaluated. In case of out-of-control situations, the complete test run needs to be repeated.

Results

ADAMTS13 activity results are reported in IU/mL. 1 IU/mL is equivalent to 100 % ADAMTS13 activity[1].

For each run, a reference curve has to be established with ADAMTS13 activity in IU/mL (x-axis) versus extinction at 450 nm (y-axis) using a linear-linear graph plot with a best fit. Control and sample are run in two replicates and concentration is read off the reference curve. Interpretation of the results should be based on the average of the two replicates.

If there are samples with extinction coefficients higher than the extinction of the highest calibrator, samples have to be pre-diluted with reaction buffer (1+1 or 1+3) and re-tested. The measured concentration then has to be multiplied with the dilution factor 2 or 4, respectively.

If the discrepancy between two replicates of a sample is higher than expected (CV >20%), the sample should be re-tested.

The assay results should be used with a cut-off of 0.1 IU/mL for TTP as recommended by the International Society on Thrombosis and Haemostasis (ISTH)[1.] in conjunction with other information, including the patient's medical history, clinical and laboratory findings, when being interpreted for diagnostic purposes.

Limitations / Interfering Substances

Interferent	Interferent test conc. (in mg/dL unless otherwise indicated)	no interference detected up to (in mg/dL unless otherwise indicated)
Acetaminophen	15.6	*
Acetylcysteine	15.0	*
Ampicillin Na	7.5	*
ASA	3.0	*
Biotin	0.351	*
Caplacizumab	0.15	*
Cefoxitin Na	660.0	*
Cyclosporine	0.18	*
Doxycycline	1.8	*
Heparin	330 units/dL	*
Ibuprofen	21.9	*
Levodopa	0.75	*
Methyldopa	2.25	*
Metronidazole	12.3	*
Phenylbutazone	32.0	*
Prednisolone	0.12	*
Rifampicin	4.8	*
Rituximab	50.0	*
Theophylline	6.0	*
human anti mouse antibody	titer >12	*
Rheumatoid factor	156 IU/mL	*
Intralipid™	2000	500
Hemoglobin	1000	220
Bilirubin	66.0	*
GST	0.02	*
VWF	2.0 IU/mL	*

*no interference tested up to highest test concentration

Performance characteristics

Performance data are given below. Results obtained in individual laboratories may differ. Precision and reproducibility have been analyzed both quantitative according to CLSI EP05-A3[4].

Precision

Lot to lot precision was assessed using 9 citrated plasma samples. Each sample was tested using three lots of reagents in two replicates per run over five days with two runs per day, resulting in a total of 30 results per level.

			Repeatability		Between Run		Between Day		Between Lot		Within Laboratory	
Sample	N	Mean (IU/mL)	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
A	30	0.65	0.041	6.3%	0.044	6.7%	0.023	3.6%	0.000	0.0%	0.050	7.6%
B	30	0.45	0.014	3.2%	0.039	8.8%	0.000	0.0%	0.014	3.0%	0.042	9.3%
C	30	0.24	0.011	4.6%	0.010	4.1%	0.002	1.0%	0.004	1.7%	0.011	4.5%
D	29	0.19	0.005	2.6%	0.009	4.6%	0.000	0.0%	0.004	2.1%	0.009	5.0%
E	30	0.14	0.004	3.1%	0.007	5.3%	0.000	0.0%	0.000	0.0%	0.007	5.3%
F	30	0.08	0.002	2.9%	0.006	8.4%	0.000	0.0%	0.000	0.0%	0.006	8.4%
G	30	0.65	0.045	6.9%	0.040	6.2%	0.019	2.9%	0.006	0.9%	0.045	6.9%
H	30	0.23	0.010	4.3%	0.014	6.2%	0.000	0.0%	0.002	0.8%	0.014	6.3%
I	30	0.12	0.004	3.7%	0.006	4.9%	0.000	0.0%	0.000	0.0%	0.006	4.9%

Operator to Operator precision was assessed using 9 citrated plasma samples. Each sample was tested by three operators in two replicates per run over five days with two runs per day, resulting in a total of 30 results per level.

			Repeatability		Between Run		Between Day		Between Operator		Within Laboratory	
Sample	N	Mean (IU/mL)	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
A	30	0.67	0.039	5.9%	0.044	6.6%	0.032	4.8%	0.012	1.9%	0.055	8.3%
B	30	0.45	0.014	3.2%	0.045	10.0%	0.000	0.0%	0.006	1.3%	0.045	10.1%
C	29	0.24	0.010	4.1%	0.012	5.2%	0.000	0.0%	0.005	2.3%	0.013	5.7%
D	29	0.19	0.006	3.1%	0.011	5.8%	0.000	0.0%	0.004	2.1%	0.012	6.2%
E	30	0.13	0.003	2.5%	0.005	4.1%	0.004	3.4%	0.003	2.5%	0.008	5.8%
F	30	0.07	0.003	3.8%	0.005	6.6%	0.000	0.0%	0.003	4.1%	0.006	7.7%
G	30	0.65	0.045	6.8%	0.045	6.8%	0.000	0.0%	0.012	1.8%	0.046	7.1%
H	30	0.23	0.007	3.2%	0.012	5.4%	0.004	1.7%	0.004	1.6%	0.013	5.9%
I	30	0.12	0.003	2.9%	0.007	5.6%	0.001	1.2%	0.003	2.4%	0.007	6.2%

Reproducibility:

Reproducibility study was conducted at three sites using the same 9 citrated plasma samples. Each sample was tested in two replicates per run over five days with two runs per day, resulting in a total of 30 results per level.

			Repeatability		Between Run		Between Day		Between Site		Reproducibility	
Sample	N	Mean (IU/mL)	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
A	30	0.68	0.046	6.8%	0.053	7.8%	0.044	6.6%	0.000	0.0%	0.069	10.2%
B	30	0.46	0.030	6.6%	0.044	9.6%	0.000	0.0%	0.010	2.2%	0.045	9.9%
C	30	0.25	0.013	5.3%	0.019	7.6%	0.000	0.0%	0.012	4.8%	0.022	9.0%
D	29	0.19	0.012	6.3%	0.016	8.6%	0.000	0.0%	0.000	0.0%	0.016	8.6%
E	30	0.14	0.008	5.9%	0.013	9.9%	0.000	0.0%	0.000	0.0%	0.013	9.9%
F	30	0.07	0.005	6.4%	0.006	7.7%	0.000	0.0%	0.005	7.0%	0.007	10.4%
G	30	0.66	0.042	6.3%	0.048	7.2%	0.014	2.0%	0.026	3.9%	0.056	8.5%
H	30	0.23	0.014	6.1%	0.016	6.9%	0.000	0.0%	0.000	0.0%	0.016	6.9%
I	29	0.12	0.005	4.2%	0.009	7.3%	0.002	1.9%	0.005	4.2%	0.010	8.7%

Clinical Performance Study:

A study was conducted at two external sites to compare the accuracy of the Technozym ADAMTS13 Activity assay relative to the clinical diagnosis of TTP. Aliquots of frozen human citrated plasma samples from individuals with TMA were tested in the Technozym ADAMTS13 activity assay. Based on a 0.1 IU/mL cut off to diagnose TTP, assay performance parameters were calculated.

		Technozym ADAMTS13 Activity		
		Negative	Positive	Total
Clinical diagnosis of TTP	Negative	101	3	104
	Positive	5	28	33
	Total	106	31	137

Sensitivity:	84.8% (28/33)	95% CI: 69.1% to 93.3%
Specificity:	97.1% (101/104)	95% CI: 91.9% to 99.0%
PPV:	90.2% (28/31)	95% CI: 75.2% to 96.6%
NPV:	95.3 (101/106)	95% CI: 90.0% to 97.8%

Bibliography

[1] Zheng XL, Vesely SK, Cataland SR, et al. ISTH guidelines for the diagnosis of thrombotic thrombocytopenic purpura. *J Thromb Haemost*. 2020;18(10):2486-2495.

[2] Clinical and Laboratory Standards Institute (CLSI). Collection, Transport and Processing of Blood Specimens for Testing Blasma-Based Coagulation and Molecular Hemostasis Assays; Approved Guideline, Fifth Edition, CLSI/NCCLS Document H21-A5; Vol. 28 No. 5.

[3] Hubbard AR, Heath AB, Hremer Hovinga JA; Subcommittee on von Willebrand Factor. Establishment of the WHO 1st International Standard ADAMTS13, plasma (12/252): communication from the SSC of the ISTH. J Thromb Haemost. 2015; 13:1151-1153.

[4] Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition. CLSI document EP05-A3.

Symbols

The following symbols and signs are used in accordance with ISO 15223-1:


Manufacturer


Batch code


Temperature limitation

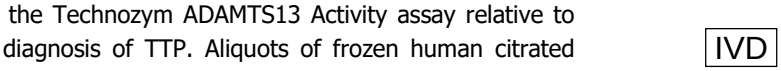

Consult instructions for use


Contains sufficient for <n> tests


Use by


Catalogue Number


Biological risks


In vitro diagnostic medical device


US Only: Federal law restricts this device to sale by or on the order of a physician.

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A point (period/stop) is always used in this Instructions for use as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

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