ENG

REF 5450401 **TECHNOZYM ADAMTS13 INH ELISA 96 T.**

REF 5450451 **TECHNOZYM ADAMTS13 INH ELISA 48 T.**

REF 5450461 **TECHNOZYM ADAMTS13 INH CAL Set 5 x 0.5 mL**

REF 5450463 **TECHNOZYM ADAMTS13 INH CONT Set 2 x 0.5 mL**

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RUO

TECHNOZYM ADAMTS13 INH ELISA - English

INTENDED USE

The TECHNOZYM ADAMTS13 INH ELISA is a quantitative test for detection of human autoantibodies (IgG) in serum or plasma against ADAMTS13, using venous-drawn fresh and/or frozen human citrated (3.2 % sodium citrate) platelet poor plasma. The chromogenic assay is performed on microplate readers capable of reading a wavelength of 450 nm. ADAMTS13 inhibitor test makes it possible to differentiate between congenital (gene polymorphisms) and acquired (autoantibodies) TTP when coupled to an activity assay and to control efficacy of plasma exchange therapy. The TECHNOZYM ADAMTS13 INH ELISA is intended for prescription use in laboratories by professionals, qualified to perform ELISA-based assays.

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 INH ELISA Kit contains:

	Reagent / Content	Description
6 x 8 wells for 48 T. 12 x 8 wells for 96 T	ELISA test strips	Microtiterplate coated with a recombinant form of ADAMTS13 protease; the drying agent is supplied in an aluminium bag.
5 x 0.5 mL	Calibrator plasma*	Numbered from 1 to 5; lyophilized, with lot-specific concentrations (values see batch table)
2 x 0.5 mL	Control plasma**	High and low control plasma, lyophilized, with lot- specific concentrations (values see batch table)
1 x 90 mL	Incubation buffer (=sample dilution buffer)	Contains stabiliser protein; 0.05 % proclin and dye; liquid; ready to use
1 x 0.3 mL	Conjugate	Anti-human IgG POX, dyed blue; liquid
1 x 12 mL	Chromogenic substrate TMB	Tetramethylbenzidine substrate; liquid, ready to use
1 x 80 mL	Washing buffer concentrate	Contains detergent; 0.01 % merthiolate, liquid
1 x 12 mL	Stop solution	Sulphuric acid 2.5 %, liquid, ready to use
2 pcs	Adhesive film	For ELISA test strips

*Additionally, the calibrators are sold separately as TECHNOZYM ADAMTS13 INH CAL Set (REF 5450461).

**Additionally, the controls are sold separately as TECHNOZYM ADAMTS13 INH CONT Set (REF 5450463).

Material required (not supplied with the kit)

- Distilled water
- Test tubes for diluting samples
- Measuring cylinder (1000 mL)
- Precision pipettes (10,100 and 1000 µL)
- Variable pipette (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
Incubation buffer / sample dilution buffer	After opening	2...8 °C	2 months
Conjugate	After opening	2...8 °C	6 months
	Working solution	Room temperature (18…25 °C)	60 minutes
Chromogenic substrate TMB	After opening	2...8 °C	Expiry date
Wash Buffer (10-fold concentrate)	After opening	2...8 °C	6 months
Washing Buffer	1+11.5 dilution of concentrate	2...8 °C	3 weeks
Stop Solution	After opening	2...8 °C	Expiry date

TEST PROCEDURE

Preparation of plasma samples

Sample material: Human serum or citrated plasma.

Citrated plasma: 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 maybe 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.

- *Sample dilution:* Dilute 1 part by volume sample with 100 parts by volume incubation buffer (1+100).
  Example: 10 µL sample + 1000 µL incubation (=sample dilution) buffer.

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.
- *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 controls are used undiluted. Reconstituted components are clear to slightly turbid.
  When freezing calibrators or control plasmas the minimum volume should be 150 µL!
- *Conjugate working solution:* Dilute 1 part by volume conjugate with 50 parts by volume incubation buffer (1+50).

Performance of the test

SAMPLE INCUBATION (reference 1,2,8)	Pipette calibrators, control plasmas, diluted samples into test wells	100 µL
	Incubate at room temperature	60 minutes
WASHING (reference 1,3,4)	Washing buffer	3 x 200 µL
CONJUGATE REACTION (reference 1,2,8)	Pipette conjugate working solution into wells	100 µL
	Incubate at room temperature	60 minutes
WASHING (reference 1,3,4)	Washing buffer	3 x 200 µL
SUBSTRATE REACTION (reference 1,2,8)	Pipette Substrate solution into test wells	100 µL
	Incubate at room temperature	10 minutes
STOPPING (reference 1,2)	Pipette stopping solution into wells	100 µL
MEASURING (reference 7)	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.
   - 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.
   - Use incubation buffer from actual kit box, do not use incubation buffer left from previous boxes. Keep incubation buffer free from contaminants.
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. No agitation is required during each reaction step.
7. By measuring the difference in wavelength at 450 nm and 620 nm, the precision of the test is increased.
8. For every incubation step, the test plate has to be covered with plate sealer.

LIMITATION OF THE TEST

Samples with high concentrations of other than anti ADAMTS13 autoantibodies may result in weak positive or borderline results.

INTERPRETATION OF RESULTS

TECHNOZYM ADAMTS13 Inhibitor results are reported in U/mL.

CALCULATION OF RESULTS

Setting up a reference curve:

X axis: ADAMTS13 IgG [U/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 on the basis of 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

negative samples. < 12 U/mL

borderline: 12 – 15 U/mL

positve samples: > 15 U/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 (in series and day to day). The following results were obtained.

	Intra assay variation		Inter assay variation	
Sample	Sample 1	Sample 2	Sample 3	Sample 4
N	24	24	20	20
Mean (U/mL)	62.9	31.2	75.4	12.8
D (U/mL)	4.9	0.9	2.7	0.7
CV (%)	7.86%	2.78%	3.54%	5.52%

Limit of detection and assay range

When the assay is performed as indicated in section 'Performance of the Test', the limit of detection of this assay is 1.68 IU/mL ADAMTS13 inhibitor. 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 are calibrated against a plasma with a very high titer of anti ADAMTS13 IgG. A 1:200 dilution of this reference plasma is defined to contain an antibody concentration of 100 U/mL (arbitrary units).

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

 Storage temperature

 Expiry date

 Consult instructions for use

 Biological

WASH Washing solution concentrate

INC Incubation buffer

SUB substrate

STOP stop solution

RTU Ready to use

RUO Research use only

LOT lot

REF Catalogue

GTIN Global Trade Item Number

BUF Reaction buffer

CAL Calibrator

CONT Control

CONJ Conjugate

MTP Microtiter plate

 Determinations

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