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TECHNOZYM® ADAMTS-13

For Research Use Only

GB DE FR

REF	5450501	TECHNOZYM® ADAMTS-13	Σ 2x48
REF	5450551	TECHNOZYM® ADAMTS-13	Σ 48
REF	5450561	TECHNOZYM® ADAMTS-13 Calibrator Set	5 x 0.5 mL
REF	5450563	TECHNOZYM® ADAMTS-13 Control Set	2 x 0.5 mL

Symbols key / Symbolschlüssel / clé des symboles

	manufactured by / Hergestellt von / fabriqué par	INC	incubation buffer / Inkubationspuffer / tampon d'incubation
	expiry date / Verfallsdatum / date d´expiration		
	storage temperature / Lagertemperatur / température de conservation	LOT	lot / Charge / lot
	consult instructions for use / Gebrauchsanweisung beachten / consulter la notice d´utilisation	MTP	microtiter plate / Mikrotiterplatte / microplaques sensibilisées
		REF	catalogue number / Katalognummer / référence
Σ	Determinations / Bestimmungen / déterminations	RTU	ready to use / gebrauchsfertig / prêt à l´emploi
AQUA	distilled Water / destilliertes Wasser / eau distillée	SUB	substrate / Substrat / substrat
CONJ	conjugate / Konjugat / conjugate	STOP	stop solution / Stopplösung / solution d´árrêt
CONT	control / Kontrolle / contrôle	WASH	washing solution concentrate / Waschlösungskonzentrat / concentrado de solución de lavado
CAL	calibrator / Kalibrator / calibrateur	STAB	stable peroxide solution / Stabile Peroxidlösung / solution stable de peroxyde
DIL	dilute or dissolve in / verdünnen oder lösen in / à diluer ou à dissoudre		

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TECHNOZYM® ADAMTS-13

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PRODUCT DESCRIPTION

INTENDED USE

The TECHNOZYM® ADAMTS-13 ELISA is a test for the determination of ADAMTS-13 activity and antigen concentration in human plasma. ADAMTS-13 is the enzyme that cleaves vWF under laminar flow conditions. A functional defect of this enzyme leads to the presence of higher molecular weight forms of vWF and thus to increased platelet aggregation, mainly in the microvasculature. This is believed to be the major cause for thrombotic thrombocytopenic purpura (TTP).

COMPOSITION

1. ELISA test strips (1x6 for 48T. and 2x6 for 2x48T.), with 8 wells each, coated with a monoclonal anti ADAMTS13, directed against the CUB domain; the drying agent is supplied in an aluminium bag.
2. Washing buffer concentrate (PBS; pH 7.3); containing detergent; 0.01% merthiolate; 1 vial, 90 mL.
3. Incubation buffer (PBS; pH 7.3); contains stabiliser protein; 0.05% proclin; and dye, 1 vial, 80 mL, ready for use.
4. Calibrators (Standards) numbered from 1 to 5; lyophilised; 1 vial each; 0.5 mL. Concentrations are lot-specific; consult the label on the vial or the batch table.
5. High and low control plasma; lyophilised; 1 vial each; 0.5 mL. Concentrations are lot-specific; consult the label on the vial or the batch table.
6. Activity substrate; 1 vial for 48T., 2 vials for 2x48T.; 3 mL each, lyophilised;
7. Conjugate: anti-ADAMTS-13 POX; dyed blue; 1 vial, 0.3 mL.
8. Antigen substrate: 1 vial, 6 mL,
9. Stable peroxyde solution; 1 vial, 0.7 mL
10. Stop solution for antigen substrate: 1 vial; 6 mL; ready to use.
11. Adhesive film: for ELISA test strips; 2 pieces.

MATERIAL REQUIRED (not supplied with the kit)

1. Distilled water
2. Measuring cylinder (1000 mL)
3. Precision pipettes (50, 100 and 1000 µL)
4. Variable pipette (100 and 1000 µL)
5. Multichannel and/or dispensing pipettes (100 and 200 µL)
6. ELISA washer
7. 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.

WARNING AND PRECAUTIONS

- 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 (see labels on the vials). However, all human blood products should be handled as potentially infectious material.
- Stopping solution for Antigen Substrate may irritate the skin. Should acid get into your eyes, wash out immediately with water and consult a doctor.
- The reagents sometimes contain preserving agents (merthiolate). Beware of swallowing! Avoid contact with skin or mucous membranes!

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
Calibrators, control plasmas	after reconstitution	-20 °C	6 months
ELISA test strip	after opening	2 ... 8 °C with adhesive film in plastic bag with drying agent	expiry date
Washing buffer conc.	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	60 minutes
Antigen substrate	After opening	2 ... 8 °C	2 years
Stable peroxide solution	After opening	2 ... 8 °C	2 years
Antigen substrate working solution	9+1 mixture of substrate and stable peroxide solution	room temperature	24 hours
Antigen substrate stop solution	After opening	2 ... 8 °C	2 years

TEST PROCEDURE

PREPARATION OF THE SAMPLES

Sample material: citrated human plasma. 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.

PREPARATION OF REAGENT

1. Before starting the test, all the required components are to be brought to room temperature.
2. Please note: maximum 6 strips (48T.) can be measured per assay.
3. Preparing the 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.
4. 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).

   No dilution is necessary for reconstituted calibrators and controls.

   Reconstituted components are clear to slightly turbid.
5. Samples are used undiluted
6. Reconstituting **activity substrate**: activity substrate is reconstituted with **3 mL** distilled water and mixed for 10 seconds after a reconstitution time of 15 minutes stored in the dark (vortex mixer)

   No dilution is necessary for reconstituted activity substrate.

   The activity substrate has to be used immediately after end of reconstitution time.
7. Preparing the conjugate working solution (1+50): Dilute 1 part by volume conjugate with 50 parts by volume incubation buffer.

   For 8 test wells: Mix 10 µL conjugate with 500 µL incubation buffer
8. Prepare **antigen substrate** working solution (9+1): mix 9 parts antigen substrate with 1 part stable peroxide solution

   For 8 test wells: Mix 450 µL antigen substrate with 50 µL stable peroxide solution

PERFORMANCE OF THE TEST

SAMPLE INCUBATION (reference 1,2,3,5, 10,13)	Pipette **calibrators, control plasmas**, **samples** into test wells; cover test strips with film	**50 µL**
	Incubate at **room temperature**	**120 minutes (2 hours)**
WASHING (reference 1,3,4)	**Washing buffer**	**3 x 200 µL**
ACTIVITY MEASUREMENT (reference 1,2,6,9,11)	**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)	Pipette **conjugate working solution** into wells, cover test strip with film	**50 µL**
	Incubate at **room temperature**	**60 minutes**
WASHING (reference 1,3,4)	**Washing buffer**	**3 x 200 µL**
ANTIGEN-SUBSTRATE REACTION (reference 1,2,8)	Pipette **antigen substrate working solution** into test wells, cover test strip with film	**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 7,12)	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
   - Test calibrators, controls and samples in duplicates
   - Incubate at indicated temperature (RT: room temperature, 20...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. 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.
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 ADAMTS-13 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 320-360nm and for emission 440-460nm.
    For BioTek reader FLx800 TBI: 360/460nm for excitation/emission.
12. Antigen measurement: suitable wavelength range for excitation 315-340nm and for emission 370-470nm.
    For BioTek reader FLx800 TBI: 360/460nm for excitation/emission.
13. A calibration curve has to be created for every assay

LIMITATION OF THE TEST

- It can not be excluded that certain forms of ADAMTS-13 (with mutations in the CUB domains) are not equivalently measured due to reduced binding to the capture antibody on the plate.
- As ADAMTS13 is a metalloprotease, EDTA plasma exhibits a significant loss of activity.
- Thrombin is reported to degrade ADAMTS13. Therefore serum samples should be avoided.
- 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.

ANALYSES RESULTS

CALCULATION OF THE RESULTS

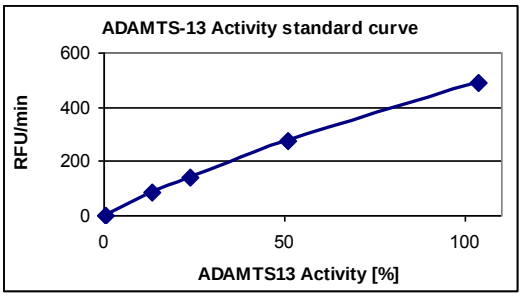
Activity Determination

Setting up a reference curve: X axis: ADAMTS13 Activity [%]
Y axis: RFU/min (slope of kinetic curve)
Graph plot is linear-linear with a cubic spline or point to point fit.

Assessment of reference curve:

- The validity of the test may be checked on the basis of the calculated values for high and low control.
- Coefficients of variation of duplicates shouldn't exceed 15%

Example of standard curve



* Y-axis scaling can be different dependent on the fluorescence reader used. This calibration curve was generated using a BioTek FLx800 TBI.

Measuring concentration of samples

- Read off the concentration from the reference curve
- If there are samples with RFU/min higher than that of the highest point of the curve, they have to be prediluted with incubation buffer (1+1 or 1+3). The measured concentration then has to be multiplied with the dilution factor 2 or 4, respectively.

Antigen Determination

Setting up a reference curve:

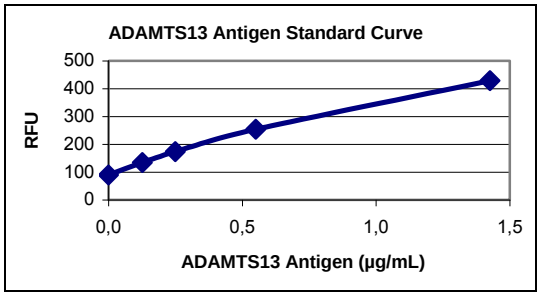
- X axis: concentration ADAMTS-13 antigen [µg/mL]
- Y axis: relative fluorescence units (RFU)

Graph plot is linear-linear with a cubic spline, linear or point to point fit.

Assessment of reference curve

- The validity of the test may be checked on the basis of the calculated values for high and low control.
- Coefficients of variation of duplicates shouldn't exceed 15%

Example of standard curve



* Y-axis scaling can be different dependent on the fluorescence reader used. This calibration curve was generated using a BioTek FLx800 TBI reader.

Measuring concentration of samples

- Read off the concentration from the reference curve.
- If there are samples with relative fluorescence units (RFU) higher than that of the highest point on the curve, they have to be prediluted with incubation 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® ADAMTS-13 evaluation software" for both, Activity and Antigen determination will be provided on request (sales@technoclone.com) or as download under www.technoclone.com.
It is recommended to use this evaluation software in combination with the BioTek reader FLx800 TBI (equipped with KC4 or Gen 5 software).

REFERENCE RANGE

Normal range for ADAMTS-13 Activity: 50-150% (n=142)
Normal range for ADAMTS-13 Antigen concentration: 0.60 – 1.60 µg/mL (n=159)

Normal range can vary depending on local population. It is recommended that individual laboratories establish their own normal. When interpreting the serological results the history of the patient has to be taken into account.

STANDARDISATION

For ADAMTS13 activity, calibrators and controls are calibrated against a pool of at least 300 normal donors defined as having 100% ADAMTS13 activity.
For ADAMTS13 Antigen concentration, calibrators and controls are calibrated against purified recombinant ADAMTS13 protein diluted in ADAMTS13 depleted plasma.

LITERATURE

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