3050192-011 02/2011

TECHNOZYM® ADAMTS-13 Activity

GB

DE

REF	5450701	TECHNOZYM® ADAMTS-13 Activity	Σ 96
REF	5450761	TECHNOZYM® ADAMTS-13 Activity Calibrator Set	6 x 0.5 mL
REF	5450763	TECHNOZYM® ADAMTS-13 Activity Control Set	2 x 0.5 mL

Symbols key / Symbolschlüssel / interpretazione dei simboli / explicación de símbolos / explicação dos símbolos

	manufactured by / Hergestellt von / prodotto da / fabricado por / fabricado por	**CAL**	Calibrator / Kalibrator / Calibratore / calibrador / calibrador
	expiry date / Verfallsdatum / data di scadenza / fecha de caducidad / data de validade	**DIL**	dilute or dissolve in / verdünnen oder lösen in / a diluire o a sciogliere in / diluir o disolver en / diluir ou dissolver em
	storage temperature / Lagertemperatur / temperatura di conservazione / limitación de temperatura / limites de temperatura	**INC**	incubation buffer / Inkubationspuffer / tampone di incubazione / tampón de incubatción / tampão de incubação
	consult instructions for use / Gebrauchsanweisung beachten / consultare le istruzioni per l'uso / consultar instrucciones de uso / consultar as instruções de utilização	**LOT**	lot / Charge / lotto / lote / lote
	Determinations / Bestimmungen / determinazioni / determinaciones / determinações,	**MTP**	microtiter plate / Mikrotiterplatte / placa microtiter / microplaca / microplaca
AQUA	distilled Water / destilliertes Wasser / acqua distillata / agua destilada / Água destilada	**REF**	catalogue number / Katalognummer / numero di catalogo / número de catálogo / referência
BUF	Reaction buffer / Reaktionspuffer / tampone di reazione / tampón de reacción / Tampão de reacção	**RTU**	ready to use / gebrauchsfertig / pronto all'uso / listo para usar / pronto a usar
WASH	washing solution concentrate / Waschlösungskonzentrat / concentrado de solución de lavado / solución de lavado concentrada / tampão de lavagem concentrado	**STOP**	stop solution / Stopplösung / Soluzione di arresto / solución de parada / solução de paragem
CONJ	Conjugate / Konjugat / Coniugato / conjugado / conjugado	**SUB**	substrate / Substrat / substrato / substrato / substrato
CONT	Control / Kontrolle / controllo / control / controlo		

Technoclone GmbH
Brunner Str. 67
1230 Vienna, Austria
www.technoclone.com

TECHNOZYM® ADAMTS-13 Activity

GB

PRODUCT DESCRIPTION

INTENDED USE (FOR RESEARCH USE ONLY)

The TECHNOZYM® ADAMTS-13 Activity ELISA is a chromogenic test for the determination of ADAMTS-13 activity in human plasma. ADAMTS-13 (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 TTP (thrombotic thrombocytopenic purpura).

COMPOSITION

1. ELISA test strips (12), with 8 wells each, coated with a monoclonal anti GST- Antibody. The drying agent is supplied in an aluminium bag.
2. GST-vWF73 Substrate; 2 vials; lyophilized; 6 mL.
3. Calibrators (Standards) numbered from 1 to 6; lyophilized; 1 vial each; 0.5 mL. **Concentrations are lot-specific; consult the label on the vial**.
4. High and low control plasma, lyophilized, 1 vial each, 0.5 mL. **Concentrations are lot-specific; consult the label on the vial.**
5. Reaction buffer; 1 vial; 30 mL; ready to use
6. Conjugate: HRP conjugated monoclonal anti-N10 Antibody: 1 vial; 12mL; ready to use
7. Colour reagent TMB (Tetramethylbenzidine); 1 vial; 12mL; ready to use
8. Wash Buffer concentrate, 10-fold concentrated, 1 vial, 53 mL.
9. Stop solution; sulphuric acid 0.5 mol/L, 1 vial, 12 mL; ready to use
10. Sample dilution Microplate, 1 plate (ONLY for sample dilution!)
11. Plate Sealer, 2 pieces.

MATERIAL REQUIRED (not supplied with the kit)

1. Distilled water
3. Measuring cylinder (500 mL)
4. Precision pipettes (5, 50, 100 and 1000 µL)
5. Variable pipette (200 and 1000 µL)
6. Multichannel and/or dispensing pipettes (100 and 200 µL)
7. ELISA washer or multichannel pipette
8. ELISA reader with 450 nm filter, with a 620 nm reference filter if available.

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 ½ Ab and HCV-Ab-negative (see labels on the vials). However, all human blood products should be handled as potentially infectious material.
- Stopping solution (sulphuric acid) 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. 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
ELISA test strip	after opening	2 … 8 °C with adhesive film in plastic bag with drying agent	Expiry date
GST-vWF73 Substrate	After reconstitution	-20 °C	6 weeks
Calibrators, control plasmas	after reconstitution	-20 °C	6 months
Reaction Buffer	After opening	2 ... 8 °C	6 months
Conjugate	After opening	2 ... 8 °C	4 months
Colour Reagent TMB	After opening	2 ... 8 °C	Expiry date
Wash Buffer (10-fold concentrate)	After opening	2 ... 8 °C	6 months
Wash Buffer	1+9 dilution of concentrate	2 ... 8 °C	3 weeks
Stop solution	After opening	2 ... 8 °C	Expiry date

TEST PROCEDURE

PREPARATION OF THE SAMPLES

Sample material: citrated human plasma. Samples may be stored up to two 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. 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.
3. Reconstituting vWF73 Substrate Solution: Substrate Solution is reconstituted with 6mL distilled water and mixed for 10 seconds after a reconstitution time of 15 minutes (vortex mixer).
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).
   Reconstituted components are clear to slightly turbid.
5. Sample / Calibrator / Control dilution: dilute samples, calibrators and controls 31-fold with reaction buffer in a sample dilution Microplate
   Example: 150µL Reaction buffer + 5µL Sample / Calibrator / Control
   For higher precision, volumes can be upscaled, using larger tubes for dilution:
   e.g. 600µL reaction buffer + 20µL Sample / Calibrator / Control

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	**100µL**
	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 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 (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. 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 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 ADAMTS13 activity, a calibration curve with calibrators 2, 3, 4, 5 and 6 is adequate.

LIMITATION OF THE TEST

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

ANALYSES RESULTS

CALCULATION OF THE RESULTS

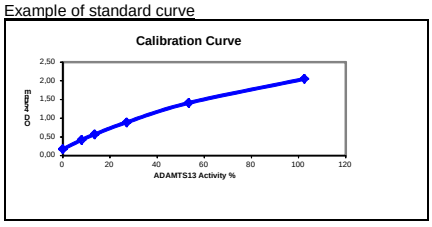
Setting up a reference curve: X axis: ADAMTS13 Activity [%]
Y axis: Extinction at 450nm

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

Assessment of reference curve:

- The extinction coefficient of the highest calibrator should be between 1.0 and 2.5.
- The extinction coefficient of the lowest Calibrator should be <0.2
- The validity of the test may be checked on the basis of the calculated control values.

Example of standard curve



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: **40 – 130 % (n=40)**

It is recommended that individual laboratories establish their own normal range. When interpreting the serological results the history of the patient has to be taken into account.

STANDARDISATION

Standards and Controls were produced from a normal donor. They were calibrated against an internal reference standard, which was produced from a pool of 300 normal donors, and defined with an activity of 100%.

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	40	40	10	10
Mean (% activity)	71,3	8,6	68,7	8,2
SD (% activity)	3,8	0,4	4,7	0,7
CV (%)	5,4	5,2	6,8	8,0

ASSAY RANGE

0,3 % – 105 %

DETECTION LIMIT

0,2 %

LITERATURE

Please contact your local distributor or Technoclone.