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# TECHNOZYM® ADAMTS-13 INH

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For Research Use Only

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|---|---|---|---|
| REF | 5450401 | TECHNOZYM® ADAMTS-13 INH | Σ 96 |
| REF | 5450451 | TECHNOZYM® ADAMTS-13 INH | Σ 48 |
| REF | 5450461 | TECHNOZYM® ADAMTS-13 INH Calibrator Set | 5 x 0.5 mL |
| REF | 5450463 | TECHNOZYM® ADAMTS-13 INH Control Set | 2 x 0.5 mL |

**Symbols key / Symbolschlüssel / interpretazione dei simboli / clé des symboles**

| | | | |
|---|---|---|---|
| | manufactured by / Hergestellt von / prodotto da / fabriqué par | **DIL** | dilute or dissolve in / verdünnen oder lösen in / a diluire o a sciogliere in / à diluer ou à dissoudre |
| | expiry date / Verfallsdatum / data di scadenza / date d´expiration | **INC** | incubation buffer / Inkubationspuffer / tampone di incubazione / tampon d'incubation |
| | storage temperature / Lagertemperatur / temperatura di conservazione / température de conservation | | |
| | consult instructions for use / Gebrauchsanweisung beachten / consultare le istruzioni per l'uso / consulter la notice d´utilisation | **LOT** | lot / Charge / lotto / lot |
| CE | CE-mark / CE-Kennzeichnung / marchio di CE / marquage CE | **MTP** | microtiter plate / Mikrotiterplatte / placa microtiter / microplaques sensibilisèes |
| Σ | determinations / Bestimmungen / determinazioni / déterminations | **REF** | catalogue number / Katalognummer / numero di catalogo / référence |
| **AQUA** | distilled Water / destilliertes Wasser / acqua distillata / eau distillée | **RTU** | ready to use / gebrauchsfertig / pronto all'uso / prêt à l´emploi |
| **CAL** | calibrator / Kalibrator / calibratore / calibrateur | **SUB** | substrate / Substrat / substrato / substrat |
| **CONJ** | conjugate / Konjugat / Coniugato / Conjugate | **STOP** | stop solution / Stopplösung / soluzione di arresto / solution d´árrêt |
| **CONT** | control / Kontrolle / control / contrôle | **WASH** | washing solution concentrate / Waschlösungskonzentrat / concentrado de solución de lavado / concentrado de solución de lavado |

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

# TECHNOZYM® ADAMTS-13 INH

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## PRODUCT DESCRIPTION

### INTENDED USE

The TECHNOZYM® ADAMTS-13 INH ELISA is a test for detection of human autoantibodies (IgG) in serum or plasma against ADAMTS-13, the protease which is responsible for cleaving unusually large vWF (ULvWF). Most of these autoantibodies inhibit ADAMTS-13 activity and thus non-cleaved ULvWF accumulates in plasma. This is believed to be the major cause for thrombotic thrombocytopenic purpura (TTP). TECHNOZYM® ADAMTS-13 INH 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.

### COMPOSITION

1. ELISA test strips (12), with 8 wells each, coated with a recombinant form of ADAMTS-13 protease; the drying agent is supplied in an aluminium bag.
2. Washing buffer concentrate (PBS; pH 7.3); containing detergent; 0.01% merthiolate; 1 vial, 80 mL.
3. Incubation buffer (= sample dilution buffer) (PBS; pH 7.3); contains stabiliser protein; 0.05% proclin; and dye, 1 vial, 90 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.**
5. Positive and negative control plasma; lyophilised; 1 vial each; 0.5 mL. **Concentrations are lot-specific; consult the label on the vial.**
6. Conjugate: anti-human IgG POX; dyed blue; 1 vial, 0.3 mL.
7. Chromogenic substrate TMB (tetramethylbenzidine); 1 vial; 12 mL; ready to use.
8. Stopping solution sulphuric acid 0.45 mol/L; 1 vial; 12 mL; ready to use.
9. Adhesive film: for ELISA test strips; 2 pieces.

### MATERIAL REQUIRED (not supplied with the kit)

1. Distilled water
2. Test tubes for diluting samples
3. Measuring cylinder (1000 mL)
4. Precision pipettes (10, 100 and 1000 µL)
5. Variable pipette (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.

## 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 (= sample dilution buffer) | after opening | 2 ... 8 °C | 2 months |
| Conjugate | after opening | 2 ... 8 °C | 6 months |
| | working solution | room temperature | 60 minutes |
| Chromogen TMB | after opening | 2 ... 8 °C | expiry date |

## TEST PROCEDURE

### PREPARATION OF THE SAMPLES

Sample material: Human serum or citrated plasma. 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.

### 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 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.
3. 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 calibrators and controls!
   Reconstituted components are clear to slightly turbid.
4. Sample Dilution: Samples are diluted with incubation buffer **(1+100) :** Dilute 1 part by volume sample with 100 parts by volume incubation buffer

   **10 µL sample + 1000 µL incubation (=sample dilution) buffer**

5. 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 20 µL conjugate with 1000 µL incubation (=sample dilution) buffer**

### PERFORMANCE OF THE TEST

| | | |
|---|---|---|
| **SAMPLE INCUBATION** (reference 1,2) | Pipette **calibrators, control plasmas**, **diluted samples** into test wells; cover test strips with film | **100 µL** |
| | Incubate at **room temperature** | **60 minutes** |
| **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 | **100 µL** |
| | Incubate at **room temperature** | **60 minutes** |
| **WASHING** (reference 1,3,4) | **Washing buffer** | **3 x 200 µL** |
| **SUBSTRATE REACTION** (reference 1,2) | Pipette **Substrate solution** into test wells, cover test strip with film | **100 µL** |
| | Incubate at **room temperature** | **10 minutes** |
| **STOPPING** (reference 1,2) | Pipette **stopping solution** into wells | **100 µL** |
| **MEASURING** (reference 5, 6) | ELISA-Reader, **450 nm** | |

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 reaction 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- and dispensing 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; by gentle tapping, the last remnants must be removed.
5. By measuring the difference in wave lengths at 450 and 620 nm the precision of the test is increased.
6. shake 10 sec., measure within 10 min.

### 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 (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 (merthiolate). Beware of swallowing! Avoid contact with skin or mucous membranes

### LIMITATION OF THE TEST

Samples with high concentrations of other than anti ADAMTS-13 autoantibodies may result in weak positive or borderline results.

## ANALYSES RESULTS

### CALCULATION OF THE RESULTS

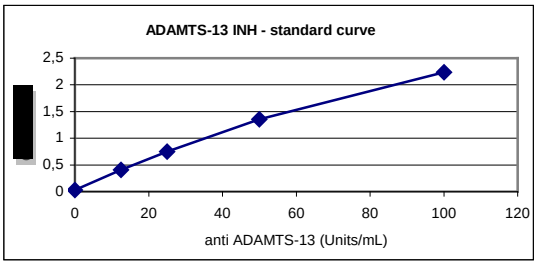
Setting up a reference curve: X axis: concentration ADAMTS-13 IgG (U/mL)
Y axis: Extinction at 450nm

Graph plot is linear-linear with a linear 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 <0.1.
- The extinction difference between these two values should be at least 1.0.
- 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 incubation buffer (1+1, or 1+3). The measured concentration then has to be multiplied with the dilution factor 2 or 4, respectively.

### INTERPRETATION OF RESULTS / REFERENCE RANGE

negative samples. < 12 units/mL
borderline: 12 – 15 units/mL
positve samples: > 15 units/mL
(n=193)

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 are calibrated against a plasma with a very high titre of anti ADAMTS-13 IgG. A 1:200 dilution of this reference plasma is defined to contain an antibody concentration of 100 Units/mL. (arbitrary units).

### 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 |
| SD (U/mL) | 4,9 | 0,9 | 2,7 | 0,7 |
| CV (%) | 7,86% | 2,78% | 3,54% | 5,52% |

**ASSAY RANGE**
2 – 104 U/mL

**DETECTION LIMIT**
1,68 U/mL

## LITERATURE


- Sadler JE. A new name in thrombosis, ADAMTS13. Proc Natl Acad Sci U S A. 2002;99: 11552-11554.
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- Fontana S, Hovinga JA, Studt JD et al. Plasma therapy in thrombotic thrombocytopenic purpura: review of the literature and the Bern experience in a subgroup of patients with severe acquired ADAMTS-13 deficiency. Semin Hematol. 2004;41:48-59.
- Klaus C, Plaimauer B, Studt JD et al. Epitope mapping of ADAMTS13 autoantibodies in acquired thrombotic thrombocytopenic purpura. Blood. 2004;103:4514-4519.