## TECHNOZYM® ADAMTS-13

**Symbols key / Symbolschlüssel / clé des symboles**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tr>
<td><img src="image" alt="Manufactured by" /></td>
<td>manufactured by / Hergestellt von / fabriqué par</td>
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<td>expiry date / Verfallsdatum / date d’expiration</td>
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<td>storage temperature / Lager temperatur / température de conservation</td>
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<tr>
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<td>consult instructions for use / Gebrauchsanweisung beachten / consulter la notice d’utilisation</td>
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<td>control / Kontrolle / contrôle</td>
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<td><img src="image" alt="Calibrator" /></td>
<td>calibrator / Kalibrator / calibrateur</td>
</tr>
<tr>
<td><img src="image" alt="Dilute or Dilute" /></td>
<td>dilute or dissolve in / verdünnen oder löschen in / à diluer ou à dissoudre</td>
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<th>Catalog Number</th>
<th>Description</th>
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<tr>
<td>5450501</td>
<td>TECHNOZYM® ADAMTS-13</td>
<td>2x48</td>
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<td>5450551</td>
<td>TECHNOZYM® ADAMTS-13</td>
<td>48</td>
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<td>5450561</td>
<td>TECHNOZYM® ADAMTS-13 Calibrator Set</td>
<td>5 x 0.5 mL</td>
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<tr>
<td>5450563</td>
<td>TECHNOZYM® ADAMTS-13 Control Set</td>
<td>2 x 0.5 mL</td>
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*GB* | *DE* | *FR*
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1230 Vienna, Austria
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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 aluminum bag.
2. Washing buffer concentrate (PBS; pH 7.3); containing detergent; 0.01% merthiolate; 1 vial, 50 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.
5. Stabilised peroxide solution.
6. Activity substrate: 1 vial for 48T; 2 vials for 2x48T.
7. Conjugate: anti-ADAMTS-13 POX; dyed blue; 1 vial, 0.3 mL.
8. Antigen substrate: 1 vial, 6 mL.
9. Stable peroxide solution; 1 vial, 0.3 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 references 11, 12. Please note that monochromators and some fluorescence reader brands are not recommended for this assay.
8. The list of available applications can be found under www.technotest.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 and samples must be considered as potentially infectious material. The activity substrate has to be used immediately after reconstitution time.
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- The reagents contain preservatives 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:

<table>
<thead>
<tr>
<th>Material/Reagent</th>
<th>State</th>
<th>Storage</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrators, control plasmas</td>
<td>after reconstitution</td>
<td>-20 °C</td>
<td>6 months</td>
</tr>
<tr>
<td>ELISA test strip</td>
<td>after opening</td>
<td>2 ... 8 °C with adhesive film in plastic bag with drying agent</td>
<td>expiry date</td>
</tr>
<tr>
<td>Washing buffer concentrate</td>
<td>after opening</td>
<td>2 ... 8 °C</td>
<td>6 months</td>
</tr>
<tr>
<td>Washing buffer</td>
<td>after opening</td>
<td>1+11.5 dilution of concentrate</td>
<td>2 ... 8 °C</td>
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<tr>
<td>Incubation buffer</td>
<td>after opening</td>
<td>2 ... 8 °C</td>
<td>2 months</td>
</tr>
<tr>
<td>Activity substrate</td>
<td>after reconstitution</td>
<td>-20 °C</td>
<td>2 months</td>
</tr>
<tr>
<td>Conjugate</td>
<td>working solution</td>
<td>2 ... 8 °C</td>
<td>room temperature</td>
</tr>
<tr>
<td>Antigen substrate</td>
<td>After opening</td>
<td>2 ... 8 °C</td>
<td>2 years</td>
</tr>
<tr>
<td>Stable peroxide solution</td>
<td>After opening</td>
<td>2 ... 8 °C</td>
<td>2 years</td>
</tr>
<tr>
<td>Antigen substrate working solution</td>
<td>9+1 mixture of substrate and stable peroxide solution</td>
<td>room temperature</td>
<td>24 hours</td>
</tr>
<tr>
<td>Antigen substrate stop solution</td>
<td>After opening</td>
<td>2 ... 8 °C</td>
<td>2 years</td>
</tr>
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</table>

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).
5. No dilution is necessary for reconstituted calibrators and controls.

REAGENT

No dilution is necessary for reconstituted activity substrate.

The activity substrate has to be used immediately after reconstitution time.

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

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. | 50 µL |

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 RU/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 RU.
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.
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 recommended 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

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