# TECHNOZYM® ADAMTS-13 Antigen

For research use only

<table>
<thead>
<tr>
<th>REF</th>
<th>Catalogue number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5450601</td>
<td>TECHNOZYM® ADAMTS-13 Antigen</td>
<td>Distilled water</td>
</tr>
<tr>
<td>5450661</td>
<td>TECHNOZYM® ADAMTS-13 Antigen Calibrator Set</td>
<td>Reaction buffer</td>
</tr>
<tr>
<td>5450663</td>
<td>TECHNOZYM® ADAMTS-13 Antigen Control Set</td>
<td>Calibrator</td>
</tr>
</tbody>
</table>

- **Symbols key**
  - Manufacturer
  - Expiry date
  - Storage temperature
  - Consult instructions for use
  - Distilled water
  - Determinations
  - Reaction buffer
  - Lot
  - Calibrator
  - Microtiter plate
  - Conjugate
  - Catalogue number
  - Control
  - Ready to use
  - Dilute or dissolve in
  - Stop solution
  - Incubation buffer
  - Substrate
  - For research use only
  - Washing solution concentrate
**TECHNOZYM® ADAMTS-13 Antigen**

**PRODUCT DESCRIPTION**

**INTENDED USE**
The TECHNOZYM® ADAMTS-13 Antigen ELISA is a chromogenic test for the determination of ADAMTS-13 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 main cause for thrombotic thrombocytopenic Purpura (TTP).

**COMPOSITION**
1. **ELISA test strips** (12), with 8 wells each, coated with a monoclonal anti-ADAMTS-13 antibody, 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, 80 mL.
3. Incubation buffer (= sample dilution buffer) (PBS; pH 7.3); contains stabilizer protein; 0.05% procain; and dye; 1 vial, 90 mL, ready for use.
4. Calibrators (Standards) numbered from 1 to 5; lyophilized; 1 vial each; 0.5 mL, Concentrations are lot-specific; consult the label on the vial.
5. High and Low control plasma: lyophilised; 1 vial each; 0.5 mL.
6. 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.
7. Stop solution sulphuric acid 0.45 mol/L; 1 vial; 12 mL; ready to use.
8. 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 (50, 100 and 200 µL)
6. ELISA washer or multichannel pipette
7. 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 the storage of the unopened vials 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 conc.</td>
<td>after opening</td>
<td>+2...8°C</td>
<td>6 months</td>
</tr>
<tr>
<td>Washing buffer</td>
<td>1+1.5 dilution of concentrate</td>
<td>+2...8°C</td>
<td>3 weeks</td>
</tr>
<tr>
<td>Incubation buffer (= sample dilution buffer)</td>
<td>after opening</td>
<td>+2...8°C</td>
<td>2 months</td>
</tr>
<tr>
<td>Conjugate</td>
<td>after opening</td>
<td>+2...8°C</td>
<td>6 months</td>
</tr>
<tr>
<td>Chromogenic substrate TMB</td>
<td>after opening</td>
<td>+2...8°C</td>
<td>expiry date</td>
</tr>
</tbody>
</table>

**TEST PROCEDURE**

**PREPARATION OF THE SAMPLES**

Sample material: Human citrated 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. 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. Preparing calibrators and control plasmas: Calibrators and control plasmas are reconstituted with 500 µL distilled water and mixed for 10 minutes after a reconstitution time of 15 minutes (vortex mixer). Reconstituted components are clear to slightly turbid.
4. Samples are used undiluted.
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 10 µL conjugate with 500 µL incubation (= sample dilution) buffer

**PERFORMANCE OF THE TEST**

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>INCUBATION (reference 1.2)</th>
<th>Washing buffer (reference 1.3.4)</th>
<th>SUBSTRATE REACTION (reference 1.2)</th>
<th>STOPPING (reference 1.2)</th>
<th>MEASURING (reference 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pipette calibrators, control plasmas, samples into test wells; cover test strips with film</td>
<td>Washing buffer 4 x 250 µL</td>
<td>Pipette conjugate working solution into wells, cover test strip with film</td>
<td>Washing buffer 4 x 250 µL</td>
<td>Pipette stopping solution into wells</td>
<td>ELISA-Reader, 450 nm</td>
</tr>
<tr>
<td>50 µL</td>
<td>120 minutes</td>
<td>60 minutes</td>
<td>15 minutes</td>
<td>50 µL</td>
<td></td>
</tr>
</tbody>
</table>

**STABILITY**
1. Reagents of different lots must not be combined.
2. Precipitation and performance, among others, depend essentially on the following factors:
   - Thorough mixing of all substrates used for dilution. 10 sec. with vortex mixer.
   - Incubate at indicated temperature (RT, 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 shall 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.
3. Labstrip 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. 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.

**LIMITATION OF THE TEST**
It can 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. Thrombin is reported to degrade ADAMTS-13. Therefore serum samples should be avoided.

**ANALYSES RESULTS**

**CALCULATION OF THE RESULTS**

Setting up a reference curve:
- X-axis: concentration ADAMTS-13 antigen [IU/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.

Example of standard curve:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>10</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (IU/mL)</td>
<td>0.55</td>
<td>0.51</td>
<td>0.49</td>
<td>0.70</td>
</tr>
<tr>
<td>SD (IU/mL)</td>
<td>0.04</td>
<td>0.01</td>
<td>0.06</td>
<td>0.01</td>
</tr>
<tr>
<td>CV (%)</td>
<td>6.36</td>
<td>5.71</td>
<td>9.97</td>
<td>6.62</td>
</tr>
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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 pre-diluted 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**

Normal range for ADAMTS-13 Antigen concentration: 0.41 – 1.41 IU/mL (n=188)

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 sample has to be taken into account.

**STANDARDISATION**

Standards and controls are produced from a pool of normal donor plasma and calibrated against the WHO International Standard for ADAMTS-13. Therefore serum samples should be avoided.

**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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Intra assay variation</th>
<th>Inter assay variation</th>
</tr>
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<tbody>
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**ASSAY RANGE**

0 IU/mL – 1.0 IU/mL (or up to the actual value of calibrator 1)

**CORRELATION**

Correlation with antigen in TECHNOZYM® ADAMTS-13 fluorogenic method is r² = 0.9 for normal samples and r² = 0.91 for TTP samples.

**LITERATURE**

Please contact Technoclone or your local distributor.