TECHNOZYM® ADAMTS-13 Activity ELISA - English

INTENDED USE

The TECHNOZYM® ADAMTS-13 Activity ELISA is a quantitative test for AATP in the research of TTP, using vero-drawn fresh and/or human blood clotted platelet poor plasma, only when compared in conjunction with other clinical findings. The assay is performed on microplate readers capable of reading a wavelength of 450 nm. The TECHNOZYM® ADAMTS-13 Activity ELISA is a chromogenic test for measurement of ADAMTS-13 activity. ADAMTS-13 activity measurement serves as an aid in the research of TTP. The TECHNOZYM® ADAMTS-13 Activity ELISA is intended for prescription use in laboratories by professionals qualified to perform ELISA-based assays.

SUMMARY

ADAMTS-13 is a protease that specifically cleaves large and small size protease inhibitor (kunitz) inhibitors, which induce platelet thrombotic formation under high shear stress. If the activity of ADAMTS13 is lowered, for some reason, however, unusually large kunitz inhibitors may accumulate within blood, causing thrombus due to platelet aggregation, which in turn may lead to TMA (thrombotic microangiopathy) such as TTP (thrombotic thrombocytopenic purpura).

REAGENTS

- ELISA test strips (12), with 8 wells each, coated with a monoclonal and GST. Antibody. The drying agent is supplied in an aluminum bag.
- GST-WF73 Substrate: 2 vials; lyophilized; 8 mL. Concentrations numbered 1 to 6; lyophilized; 1 vial each; 0.5 mL. Concentrations are lot-specific; consult the batch table.
- Additionally, the calibrators are sold separately as TECHNOZYM® ADAMTS-13 Activity Calibration Set (REF 545071).
- Reaction buffer: 1 vial; lyophilized; ready to use.
- Contagate: HRP conjugated monoclonal antibody: 1 vial; 12 mL; ready to use.
- TMB Substrate (Tetramethylbenzidine): 1 vial; 12 mL; ready to use.
- Wash buffer concentrate: 10-fold concentrated, 1 vial; 53 mL.
- Stop solution: sulfuric acid 0.5 M; 1 vial; 12 mL; ready to use.
- Sample dilution Microplate; 1 plate (ONLY for sample dilution).
- Plate sealer 3 pieces.

Material required (not supplied with the kit)

- Distilled water
- Measuring cylinder (500 mL)
- Precipitation pipettes (5, 10, 100 and 1000 µL)
- Variable pipette (200 and 1000 µL)
- Mixed buffer concentrate (10-fold diluted)
- GST-vWF73 Substrate; 2 vials; lyophilized; 6 mL.
- 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 rules. The regulations pertaining to disposal are the same as applied to disposing hospital waste.
- Calibrators and control plasmas are made from human blood and any individual involved in the procedure is HbsAg, HIV 1/2 Ab and HCV-Ab-negative as tested by FDA approved methods. However, all human blood products should be handled as potentially infectious material.
- Get a Material Safety Data Sheet for this product from www.technozyme.com

Warming and precautions

- For research use only
- The kit is intended for use by personnel trained in laboratory procedures and universal precautions for the use of chemicals and potentially biohazardous substances.
- 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 rules. The regulations pertaining to disposal are the same as applied to disposing hospital waste.
- Calibrators and control plasmas are made from human blood and any individual involved in the procedure is HbsAg, HIV 1/2 Ab and HCV-Ab-negative as tested by FDA approved methods. However, all human blood products should be handled as potentially infectious material.
- For every Incubation step test plate has to be covered with plate sealer.
- It is not mandatory to use all six calibrators for creating a calibration curve. For sample screening a calibration curve using calibrator 1, 3, 4, 5 and 6, respectively. When the focus is particularly in the lower range of ADAMTS-13 activity, a calibration curve with calibrators 2, 3, 4, 5 and 6 is adequate.
- Reproducibility was determined with different samples.
- Stability and storage

Stability

The expiry date printed on the labels applies to storage of the unopened vial at 2 to 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>ELISA test strip</td>
<td>after reconstitution</td>
<td>-20 °C</td>
<td>6 months</td>
</tr>
<tr>
<td>GST-WF73 Substrate</td>
<td>after reconstitution</td>
<td>-20 °C</td>
<td>6 months</td>
</tr>
<tr>
<td>Diluted control or samples</td>
<td>after reconstitution</td>
<td>-20 °C</td>
<td>6 months</td>
</tr>
<tr>
<td>Reaction buffer</td>
<td>after reconstitution</td>
<td>2 °C to 8 °C</td>
<td>6 months</td>
</tr>
<tr>
<td>Contagate</td>
<td>after reconstitution</td>
<td>2 °C to 8 °C</td>
<td>4 months</td>
</tr>
<tr>
<td>TMB Substrate</td>
<td>after reconstitution</td>
<td>2 °C to 8 °C</td>
<td>6 months</td>
</tr>
<tr>
<td>Stop solution</td>
<td>after stop solution</td>
<td>2 °C to 8 °C</td>
<td>6 months</td>
</tr>
</tbody>
</table>

TEST PROCEDURE

Preparation of the samples

Sample collected: humanized plasma. Blood is collected into 3.2 % Buffere Chilled anticoagulant tubes at a ratio of 9 volumes blood to 1 volume anticoagulant and gently mixed by inversion. Centrifuge at a minimum of 10 g for 10 minutes (ELISA Guideline 1.1.2) and remove supernatant plasma. Samples may be stored to up to 2 hours at room temperature. Samples may be frozen once at ≤ -20 °C.

Preparation of reagent

Before starting the test, all the required components are to be brought to room temperature.

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 5 minutes.

Reconstituting GST-WF73 Substrate: Substrate Solution is reconstituted with 1 mL distilled water and mixed for 10 minutes. Incubate at room temperature 10 minutes.

Reconstituting calibrators and control plasmas: Calibrators and control plasmas are reconstituted with 500 µL distilled water and mixed for 10 minutes. Incubate at room temperature 10 minutes (vortex mixer).

Reconstituted components are clear to slightly turbid. Sample / Calibrator / Control dilution: dilute samples, calibrators and controls 1:10 with buffer solution in a sample dilution Microplate.

Example: 150 µL Reaction buffer < 5 µL Sample / Calibrator / Control

For higher precision, volumes can be increased: using larger tubes for dilution: e.g. 800 µL Reaction buffer > 20 µL Sample / Calibrator / Control

Performance of the test

Sample INCUBATION (reference 1.2, 5, 6.7, 10)

Incorporate at room temperature 10 min.

WASHING (reference 1.3, 4)

Washing buffer 3 x 300 µL

CONJUGATE REACTION (reference 1.2, 7.9)

Incorporate at room temperature 60 min.

WASHING (reference 1.3, 4)

Washing buffer 3 x 300 µL

TMB COLOUR DEVELOPMENT REACTION (reference 1.2, 7.9)

Incorporate at room temperature 10 min.

STOPPING (reference 1.7, 2)

Pipe out substrate solution into test wells 100 µL

MEASUREMENT (reference 8)

ELISA reader, 450 nm

450 nm absorbance, measure within 10 min.

LIMITATION OF THE TEST

Samples containing EDTA cannot be used because EDTA is a strong inhibitor of ADAMTS-13 function.

Hemolysis: No interference is observed with samples containing up to 200 mg/L hemoglobin, which corresponds with a moderate to severe concentration.

Hepatitis: If interference occurs with samples containing more than 1000 IU/mL HCV-Ab, consult the manufacturer.

Lipemia: No interference is observed with samples containing up to 300 mg/dL Intralipid™, which corresponds with a moderate to severe concentration.

Rheumatoid factor: No interference was observed up to 28 IU/mL RF, with corresponds with a 2-fold concentration of unconjugated), which corresponds with a moderate to severe concentration.

Rheumatoid factor: No interference was observed up to 28 IU/mL RF, with corresponds with a 2-fold concentration of anti-C20 antibodies. No interference was observed up to a level of 200 µg/mL, which corresponds to the upper level of serum concentrations found after Rhizomia administration.

CALCULATION OF RESULTS

Setting up a reference curve:

X axis: ADAMTS-13 Activity [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 calculated control values.

Measuring concentration of samples

Read off the concentration from the reference curve

Reference range

Normal range for ADAMTS-13 Activity: 0.4 – 1.3 IU/mL

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

Performance characteristics

Sensitivity 98.73 % 93.15 % to 99.97 %

Specificity 92.86 % 80.52 % to 98.50 %

Negative Predictive Value 97.50 % 94.73 % to 99.64 %

Positive Predictive Value 98.50 % 93.15 % to 99.97 %

Precision

Reproducibility was determined with different samples.

Limit of quantification and assay range

When assay is performed as indicated in Section ‘Test Procedure’, the limit of quantification of this assay is 0.0071 IU/mL ADAMTS-13 activity. The upper limit of detection may vary with each lot of kit depending on the assayed value of the calibrator plasma supplied in the kit. Samples with values outside the range of the reference curve should be re-tested at an appropriate dilution to obtain accurate results.

LITERATURE

Please contact Technozyme or your local distributor.
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Key</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Manufacturer" /></td>
<td>RTU</td>
<td>Ready to use</td>
</tr>
<tr>
<td><img src="image" alt="Storage temperature" /></td>
<td>CAL</td>
<td>Calibrator</td>
</tr>
<tr>
<td><img src="image" alt="Expiry date" /></td>
<td>CONT</td>
<td>Control</td>
</tr>
<tr>
<td><img src="image" alt="Consult instructions for use" /></td>
<td>CONJ</td>
<td>Conjugate</td>
</tr>
<tr>
<td><img src="image" alt="Determinations" /></td>
<td>BUF</td>
<td>Reaction Buffer</td>
</tr>
<tr>
<td><img src="image" alt="Global Trade Item Number" /></td>
<td>SUB</td>
<td>Substrate</td>
</tr>
<tr>
<td><img src="image" alt="Biological risk" /></td>
<td>STOP</td>
<td>Stop solution</td>
</tr>
<tr>
<td><img src="image" alt="For research use only" /></td>
<td>WASH</td>
<td>Washing solution concentrate</td>
</tr>
<tr>
<td><img src="image" alt="Lot" /></td>
<td>MTP</td>
<td>Microwell plate</td>
</tr>
<tr>
<td><img src="image" alt="Catalogue number" /></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>