

5-ELISA ADAMTS-13 Antigen

Ref# 5D-55501

ELISA for measurement of ADAMTS-13

96 tests micro-ELISA plate



Research Use Only. Not for use in diagnostic procedures

Intended use :

The 5-ELISA ADAMTS-13 Antigen kit is a two-site enzyme immunoassay for measuring ADAMTS-13 antigen in plasma or in any biological fluid where it must be tested.

Summary and explanation:

This sandwich ELISA is designed with polyclonal antibodies coated onto the plate for capturing ADAMTS-13 in the tested sample. Following a washing step, captured ADAMTS-13 is tagged with a peroxidase-labeled polyclonal antibody, which binds onto the free epitopes in a dose-dependent manner. After washing away the excess of immunconjugate, the substrate, 3,3',5,5'-Tetramethylbenzidine (TMB) with hydrogen peroxide (H₂O₂) is introduced and a blue color develops, which turns yellow when the reaction is stopped with sulfuric acid. This color is measured at 450 nm, and the measured value is directly proportional to the amount of ADAMTS-13 present in the tested sample. Results are expressed in International Units (IU) per mL, by reference to the International Standard for ADAMTS-13 in plasma (1).

ADAMTS-13 is a metalloproteinase present in plasma at a concentration of about 500-800 ng/mL. It cleaves high molecular weight von Willebrand factor polymers to a lower molecular weight, following its release into blood circulation from endothelial cells (released from Weibel-Palade bodies) (2).

Reagents:

- COAT:** Micro ELISA plate, containing 12x8 well strips, coated with a polyclonal antibody to human ADAMTS-13, stabilized; the plate is packed in an aluminum pouch sealed in presence of a desiccant.
- SD:** 2 vials of 30 mL Sample Diluent containing aggregated rabbit IgGs, ready to use.
- WS:** 50 mL vial of 20-fold concentrated Wash Solution.
- CAL:** 2 vials of lyophilized Plasma Calibrator, **already diluted 1:20** when restored with 2 mL of SD*.
- C1 (High):** 2 vials of lyophilized Control Plasma High, **already diluted 1:20** when restored with 1 mL of SD*.
- C2 (Low):** 2 vials of lyophilized Control Plasma Low, **already diluted 1:20** when restored with 1 mL of SD*.
- IC:** 500 µL vial of Anti-(h)-ADAMTS-13-HRP Conjugate, 50-fold concentrated.
- ICD:** 25 mL of Conjugate Diluent, ready to use.
- TMB:** 26 mL of Peroxidase Substrate, 3,3',5,5'-Tetramethylbenzidine containing hydrogen peroxide. Ready to use.
- SA:** 7.5 mL vial of 0.50M Sulfuric Acid, ready to use.

* The exact ADAMTS-13 concentrations are indicated for each lot on the flyer included in the kit.

Warning and cautions:

Some reagents provided in this kit contain materials of human (control and calibration plasma) and animal origin. Whenever human plasma is required for the preparation of these reagents, approved methods are used to test the plasma for the antibodies to HIV 1, HIV 2 and HCV, and for hepatitis B surface antigen, and results are found to be negative.

The bovine blood used to prepare BSA has been tested by recorded methods and is certified free of infectious agents, in particular the causative agent of bovine spongiform encephalitis.

However, no test method can offer complete assurance that infectious agents are absent. Therefore, laboratory operators using these reagents must exercise extreme care in full compliance with safety cautions for the manipulation of these biological materials and treat them as if they were infectious.

- Waste should be disposed in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.
- Any incident that has occurred in relation with the device use shall be reported to the manufacturer.
- If the TMB substrate becomes yellow, this indicates the presence of a contaminant.

Reagent preparation:

Bring the kit at room temperature, at least 30 minutes before use, to avoid use of reagents at a too low temperature, which can reduce the assay kinetics.

Store unused reagents at 2-8°C.

Vials are closed under vacuum. Carefully remove the stopper of lyophilized reagents, to avoid any loss of powder when opening the vials.

When appropriately used and stored, according to the recommended protocol and cautions, the kit content can be used over a 1-month period, and strip by strip, if required.

- COAT** (Micro ELISA plate): Open the aluminum pouch and take off the required number of 8-well strips for the test series. When out of the pouch, the strips must be used within 30 minutes. Unused strips can be stored at 2-8°C for 4 weeks in their original aluminum pouch, in presence of the desiccant, hermetically closed with the minigrip system and protected from any moisture.
- SD** (Sample Diluent): Ready to use.
This reagent contains 0.05% Proclin-300, and aggregated rabbit IgGs to minimize the interference of Rheumatoid Factor and heterophilic antibodies. Stability after opening and provided that any contamination or evaporation is avoided, kept in its original vial:
 - 4 weeks at 2-8°C.
- WS** (Wash Solution): If necessary, incubate the vial in a water bath at 37°C, until complete dissolution of solids. Shake the vial and dilute the required volume 1:20 with distilled water (the 50 mL contained in the vial allow preparing 1 liter of Wash Solution). Stability after opening and provided that any contamination or evaporation is avoided, kept in its original vial:
 - 4 weeks at 2-8°C.
- CAL** (Calibrator): Reconstitute each vial with exactly 2 mL of **SD** sample diluent to get a 20-fold diluted calibrator plasma with a defined ADAMTS-13 concentration (indicated on the kit flyer). Stability of reconstituted calibrator, in its original vial and provided that any contamination or evaporation is avoided:
 - 8 hours at room temperature (18-25°C).
 - 24 hours at 2-8°C.
 - 2 months at ≤ -20°C.
- C1, C2** (Control Plasma 1 High and Control Plasma 2 Low): Reconstitute each vial with 1 mL of **SD** sample diluent to get a 20-fold diluted control plasma. Stability of reconstituted control, in its original vial and provided that any contamination or evaporation is avoided:
 - 8 hours at room temperature (18-25°C).
 - 24 hours at 2-8°C.
 - 2 months at ≤ -20°C.
- IC** (Anti-(h)-ADAMTS-13-HRP Conjugate): Stability after opening and provided that any contamination or evaporation is avoided, kept in its original vial:
 - 4 weeks at 2-8°C.Just before use, dilute 50-fold the requested volume of the concentrated **IC** (50x) with the **ICD** "Conjugate Diluent" and shake to homogenize. Stability of diluted Conjugate:
 - 6 hours at room temperature (18-25°C).
- ICD** (Immuno-Conjugate Diluent): Ready to use. This reagent contains 0.05% Proclin-300. Stability after opening, and provided that any contamination or evaporation is avoided, kept in its original vial:
 - 4 weeks at 2-8°C.
- TMB:** Ready to use. Stability after opening, and provided that any contamination or evaporation is avoided, kept in its original vial:
 - 4 weeks at 2-8°C.
- SA** (Stop Solution): Stop solution containing 0.50M sulfuric acid, ready to use.

Reagents and materials not provided:

- 8-channel or repeating micro-ELISA pipette for volumes of 50-300 µL.
- Pipettes at variable volumes from 0 to 20, 20 to 200 and 200 to 1000 µL.
- Micro ELISA plate reader with a wavelength set up at 450 nm.

Specimen collection and preparation:

Blood (9 volumes) should be carefully collected onto 0.109M (3.2%) trisodium citrate anticoagulant (1 volume) by clean venipuncture.

Samples (plasma or serum) should be collected, prepared, and stored in accordance with applicable local guidelines.

For sample storage, please refer to references.

Assay method:

2-step protocol:

The standard assay protocol used on plasma, includes a 1:20 dilution. Measured ADAMTS-13 concentrations in tested plasmas with these assay conditions are directly deduced from the calibration curve. If other samples are used, the plotted concentrations reported on abscissae must be divided by 20, and the measured concentration must be multiplied by the dilution factor used for the tested specimen. Tested samples must be diluted at least 1:5 with the **SD** sample diluent.

- Controls are ready to use (already diluted 1:20).
- Tested plasma samples should be diluted 1:20 (or more when required) with the **SD** sample diluent.
- Immunoconjugate **IC** must be diluted 1:50 with the **ICD** conjugate diluent, just before use.
- Calibration range: **CAL**, with an ADAMTS-13 concentration of "c", must be diluted with **SD** to get the following calibration range:

	c	0.75 c	0.50 c	0.25 c	0.10 c	0
CAL µL	500	375	250	125	50	0
SD µL	0	125	250	375	450	500

- Procedure: Remove the required number of strips from the aluminium pouch and place them in the frame provided. Introduce the reagents in the microplate wells and perform the assay as indicated on the here below table:

Samples or Reagent	Volume	Protocol
Calibrators, Controls (C1, C2) or tested specimen	200 µL/well	Introduce rapidly into microwells (a)
<i>Incubate for 60 minutes at RT (18-25 °C) (b) (c)</i>		
WS	300 µL/well	5 successive washing steps
IC	200 µL/well	Introduce immediately after washing (d)
<i>Incubate for 60 minutes at RT (18-25°C) (b) (c)</i>		
WS	300 µL/well	5 successive washing steps
TMB-H₂O₂	200 µL/well	Introduce immediately (d)
<i>Incubate for exactly 5 minutes at RT (18-25 °C) (b) (c)</i>		
SA (e)	50 µL/well	Stop after exactly 5 minutes
<i>Homogenize by shaking smoothly and wait for 10 minutes Read the absorbance at 450 nm within 20 minutes (f)</i>		

One-step protocol:

This assay protocol used on plasma includes a 1:10 dilution with **SD**. Measured ADAMTS-13 concentrations in tested plasmas with these assay conditions are directly deduced from the calibration curve. If other samples are used, the plotted concentrations reported on abscissae must be divided by 10, and the measured concentration must be multiplied by the dilution factor used for the tested specimen. Tested samples must be diluted at least 1:2 with the **SD** sample diluent.

Calibrator, controls and immunoconjugate **IC** must be used **2-fold** more concentrated than for the 2-step protocol:

1. Calibrator (**CAL**) must be restored with **1 mL SD**.
2. Controls **C1-High** and **C2-Low** must be restored with **0.5 mL SD** and are ready to use (already diluted **1:10**).
3. Tested plasmas must be diluted **1:10** (or more when required) with the **SD** sample diluent.
4. Immunoconjugate **IC** must be diluted 1:25 with the **ICD** conjugate diluent, just before use.
5. Calibration range: **CAL**, with an ADAMTS-13 concentration of "c", must be diluted with **SD** to get the following calibration range:

	c	0.75 c	0.50 c	0.25 c	0.10 c	0
CAL µL	300	225	150	75	30	0
SD µL	0	75	150	225	270	300

6. **Procedure:** Remove the required number of strips from the aluminium pouch and place them in the frame provided. Introduce the reagents in the microplate wells and perform the assay as indicated on the here below table:

Samples or Reagent	Volume	Protocol
IC	100 µL/well	Add rapidly immediately followed by:
Calibrators, Controls (C1, C2) or tested specimen	100 µL/well	Introduce rapidly into microwells (a) Shake gently for mixing
<i>Incubate for 60 minutes at RT (18-25 °C) (b) (c)</i>		
WS	300 µL/well	5 successive washings
TMB-H₂O₂	200 µL/well	Introduce immediately (d)
<i>Incubate for exactly 8 minutes at RT (18-25 °C) (b) (c)</i>		
SA (e)	50 µL/well	Stop after exactly 8 minutes
<i>Homogenize by shaking smoothly and wait for 10 minutes Read the absorbance at 450 nm within 20 minutes (f)</i>		

Notes:

- (a) Distribute controls and specimen as rapidly as possible (within 10 minutes), to obtain homogeneous immunological kinetics. A too long delay between the distribution of the first and the last wells may influence immunological kinetics and generate inaccurate results (last wells distributed underestimated).
- (b) Avoid letting the plate in the bright sunlight during incubations and more particularly during color development.
- (c) Perform the incubations preferably at 20±1°C.
- (d) Never let the plates empty between the addition of the reagents or following the washing step. The next reagent must be added within 3 minutes, to prevent the plate from drying, which could damage the immobilized components and reduce reagents' reactivity. If necessary, keep the plate filled with Wash Solution and empty it just before the introduction of the next reagent. The washing instrument must be adjusted to wash the plates gently, and to avoid a too drastic emptying, which could damage coating and lower plate reactivity.
- (e) For addition of the substrate, the time interval between each row must be accurate and exactly determined. It must be the same when stopping the reaction. For the one-step protocol the color development has lower kinetics than for the 2-step one, therefore the color development time can be extended to 8 min.
- (f) For bichromatic readings, a reference wavelength between 620 nm and 690 nm can be used.

Quality control:

Using quality controls allows validating the method compliance, as well as the homogeneity of assays for a same lot of reagents.

Quality control must be included in each series, as per good laboratory practice, to validate test results.

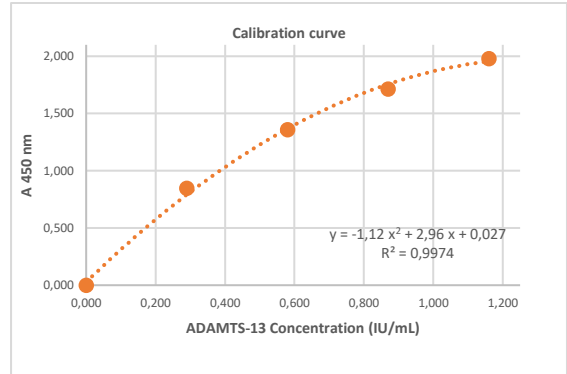
Each laboratory should establish acceptance ranges and verify expected performances in its analytical system, in case of assay automatization, the user agrees to conduct a complete study to validate the analytical performance according to the standards in force.

Results:

For the calibration curve, plot on abscissae the ADAMTS-13 concentration, and on ordinates the corresponding A450, then draw the calibration curve (best fit, or polynomial). Using the A450 obtained for the tested sample dilution, calculate the ADAMTS-13 concentration. An ELISA software is currently used for that measurement and is the recommended method.

The concentration in the assayed sample, is directly obtained, as the calibration already includes the **1:10 standard assay dilution factor**.

Example of calibration curve (must not be used for ADAMTS-13 measurements; only the calibration curve generated for the ongoing tested series must be used):



Limitations:

- To ensure optimum test performance and to meet the specifications, the technical instructions should be followed carefully.
- Any reagent presenting no limpid appearance or showing signs of contamination must be rejected.
- Any suspicious samples must be rejected.
- If the washing step is not correctly performed, this can produce a high absorbance value. To avoid non-specific color development, check washing efficiency.
- It is the responsibility of the user to validate modifications to these instructions or use of the reagents.
- Erroneous results can occur from bacterial contamination of reagents, inadequate incubation periods, inadequate washing of test wells, exposure of substrate to bright light/sunlight, omission of test reagents, exposure to temperatures higher or lower than prescribed requirements or omission of steps.
- Aggregated rabbit IgGs are added to the sample diluent (**SD**) to minimize any possible interference of Rheumatoid Factor or of heterophilic antibodies.

Performances:

Dynamic range: 0.05 to about 1.20 IU/mL ADAMTS-13 in plasma (i.e. 0.0025 to 0.060 IU/mL in the tested 20-fold dilution)

Intra-assay CV: C1 (0.87 IU/mL) 5.77%; C2 (0.47 IU/mL): 4.79%

Inter-assay CV: C1 (0.87 IU/mL) 5.32%; C2 (0.47 IU/mL): 5.92%

Recovery: 90-110 % for purified ADAMTS-13 spiked in citrated plasma.

Normal concentration in plasma:

0.60 to 1.30 IU/mL (3)

Additional information:

ADAMTS-13 is a disintegrin and metalloprotease with thrombospondin-1-like domain which cleaves large vWF multimers at endothelial cell surface. Its decreased concentration or activity leads to large vWF multimers in blood circulation, with increased risk of arterial thrombosis and a high platelet aggregation tendency. It is synthesized by hepatic and endothelial cells. This protein contains 1,427 amino acids and has a predicted molecular weight of 145 kDa.

References:

1. Hubbard AR, Heath AB, Kremer Hovinga JA; Subcommittee on von Willebrand Factor. Establishment of the WHO 1st International Standard ADAMTS13, plasma (12/252): communication from the SSC of the ISTH. J Thromb Haemost. 2015 Jun;13(6):1151-3.
2. Zheng XL. Structure-function and regulation of ADAMTS-13 protease. J Thromb Haemost. 2013 Jun;11 Suppl 1(0 1):11-23.
3. Bazzan M, Montaruli B, Sciascia S, Cosseddu D, Norbiato C, Roccatello D. Low ADAMTS 13 plasma levels are predictors of mortality in COVID-19 patients. Intern Emerg Med. 2020 Aug;15(5):861-863.

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