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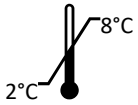
IMUBIND® ADAMTS13 ELISA

REF 813RUO

For Research Use Only



INTENDED USE

The IMUBIND® ADAMTS13 ELISA is intended for the measurement of ADAMTS13 protein in human plasma. The assay is limited to 'For Research Use Only'.

EXPLANATION OF THE TEST

ADAMTS13, also known as von Willebrand Factor (vWF) cleaving protease, is a zinc metalloproteinase that cleaves ultra large vWF multimers (UL-vWF) between Tyr(1605)-Met(1606), located in the A2 region of vWF.[1,2] A deficiency of ADAMTS13 activity (<5%) can lead to an accumulation of UL-vWF multimers.[3] The UL-vWF multimers bind to receptors on platelets inducing platelet aggregation and formation of intravascular thrombi.

Congenital TTP is a rare inherited disorder resulting from mutations in the ADAMTS13 gene. Genetic alterations have been identified at many sites within the ADAMTS13 gene, which can lead to the production of non-functional ADAMTS13 protein.[4,5] The acquired form of TTP is an autoimmune disorder caused by the development of autoantibodies to ADAMTS13 that inhibit its activity or may cause a reduction in antigen levels.[6-8]

PRINCIPLE OF THE METHOD

Diluted plasma samples are added to microwells coated with a monoclonal antibody that binds to ADAMTS13. During an incubation period, ADAMTS13 in a sample binds to the antibody coated to the wells. Following a washing step, a biotinylated rabbit anti-ADAMTS13 polyclonal antibody is added to the microwells, which binds to ADAMTS13 protein captured on the plate during a short incubation period. A streptavidin-horseradish peroxidase conjugate (SA-HRP) is added to the microwells to complete the formation of the antibody-enzyme detection complex. Following another washing step, the addition of a perborate-3,3'-5,5'-tetramethylbenzidine (TMB) substrate and its subsequent reaction with the HRP present generates a blue colored solution. The reaction is stopped by adding sulfuric acid, which turns the solution color yellow. Measuring the solution absorbance at 450 nm and extrapolating the value with those of a standard curve determines the level of ADAMTS13 in the diluted plasma sample.

REAGENTS

MAb Anti-Human ADAMTS13 Coated Microwells, 96, with acetate cover sheet
Assay Buffer, 15 mL, 3 vials (lyophilized)
ADAMTS13 Standard, 100 ng/mL, 0.6 mL, 2 vials (lyophilized)
Positive Control, 0.25 mL, 2 vials (lyophilized)
Detection Antibody, biotinylated rabbit anti-human ADAMTS13 IgG, 120 µL
Enzyme Conjugate, SA-HRP, 120 µL
Substrate, TMB, 11 mL
Wash Buffer, PBS with 0.05% Tween 20, pH 7.4, 1 L (lyophilized)

WARNINGS AND PRECAUTIONS

Source material for some of the reagents in this kit is of human origin. This material has been found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1, HIV-2) using FDA approved methods. As no known test method provides complete assurance that products derived from human blood will not transmit HBsAg, HCV, HIV-1, HIV-2 or other blood-borne pathogens, reagents should be handled as recommended for any potentially infectious human specimen. Discard all waste associated with test specimens and human source reagents in a biohazard waste container.

Limited to 'For Research Use Only' in the United States. For *in vitro* use only. Not for internal use in humans or animals. Do not use the kit components beyond the stated expiration date. Do not mix reagents from different kits. Avoid microbial contamination of the reagents. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled. Do not pipette reagents by mouth. Wear laboratory coat and disposable gloves throughout the test procedure and wash hands thoroughly afterwards. Avoid splashing or aerosol formation.

Assay Buffer	Danger		CONT: EDTA trisodium salt, Polyethylene glycol octylphenol ether H318, H412, P280, P273, P305 + P351 + P338, P310
Detection Antibody	Warning		H319, P264, P280, P305 + P351 + P338, P337 + P313
Enzyme Conjugate	Warning		CONT: 2-methyl-4-isothiazol-3-one H317, P261, P272, P280, P302 + P352, P333 + P313
Wash Buffer	Warning		H319, P264, P280, P305 + P351 + P338, P337 + P313

Hazard Statements:
H317 May cause an allergic skin reaction.
H318 Causes serious eye damage.
H319 Causes serious eye irritation.
H412 Harmful to aquatic life with long lasting effects.

Precautionary Statements:
P261 Avoid breathing mist or vapor.
P264 Wash thoroughly after handling.
P272 Contaminated work clothing must not be allowed out of the workplace.
P273 Avoid release to the environment.
P280 Wear eye protection/face protection. Wear protective gloves.
P302 + P352 If on skin: Wash with plenty of water.
P305 + P351 + P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a poison center/doctor.
P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.
P337 + P313 If eye irritation persists: Get medical advice/attention.

REAGENT PREPARATION AND STORAGE

Unopened and lyophilized reagents are stable until the expiration date printed on the box when stored as instructed.

1. Antibody Coated Microwells: Once removed from the foil pouch, the microwell strips must be used within 30 minutes. Unused strips may be stored at 2°-8°C for 4 weeks when sealed in the original pouch with the desiccant present and protected from moisture.
2. Assay Buffer: Reconstitute with 15 mL of filtered deionized/distilled water. Gently mix the contents of the vial. Assay Buffer may be used for up to 4 weeks when stored at 2°-8°C.
3. ADAMTS13 Standard: Reconstitute with 0.6 mL of Assay Buffer. Standard may be aliquoted and stored at -20°C for up to 6 months. The standard is prepared from normal plasma calibrated against a recombinant ADAMTS13.
4. Positive Control: Reconstitute with 0.25 mL of Assay Buffer. Once reconstituted, the control may be stored for up to 4 weeks at -20°C.
5. Detection Antibody: Supplied as a concentrate, dilute the Detection Antibody 1:100 with Assay Buffer just prior to use. For using all 96 microwells at one time, dilute 100 µL of Detection Antibody to 10 mL in Assay Buffer. If all 96 microwells are not to be used, dilute 20 µL of Detection Antibody to 2 mL in Assay Buffer for each 16-microwell strip that will be used. Working strength Detection Antibody is stable for 4 hours at 2°-8°C. Discard any unused working strength Detection Antibody.
6. Enzyme Conjugate, SA-HRP: Supplied as a concentrate, dilute the Enzyme Conjugate 1:100 with Assay Buffer just prior to use. For running all 96 microwells at one time, dilute 100 µL of Enzyme Conjugate to 10 mL in Assay Buffer. If all 96 microwells are not to be used, dilute 20 µL of Enzyme Conjugate to 2 mL in Assay Buffer for each 16-microwell strip that will be used. Working strength Enzyme Conjugate is stable for 2 hours at 2°-8°C. Discard any unused working strength Enzyme Conjugate.

7. Substrate, TMB: Supplied ready to use. It may be used until the expiration date stated on the vial when stored in the dark at 2°-8°C.
8. Wash Buffer: Dissolve the contents of the packet with 1 L of filtered deionized/ distilled water. After reconstitution, the Wash Buffer may be used for up to 4 weeks when stored at 2°-8°C.

SPECIMEN COLLECTION AND PREPARATION

Only citrate collected platelet poor plasma may be used for this assay. Do Not Use EDTA collected plasma. See "Collection, Transport and Processing of Blood Specimens for Testing Plasma-based Coagulation and Molecular Hemostasis Assays; Approved Guidelines-Fifth Edition", CLSI Document H21-A5, Vol. 28, No. 5, 2008. Plasma collection should be performed as follows:

1. Collect 9 parts of blood into 1 part of 3.2% (0.109 M) trisodium citrate anticoagulant solution.
2. Centrifuge the blood sample at 10,000 x g for 15 minutes.
3. Plasma should be stored at 2°-8°C and assayed within 4 hours. Alternatively, plasma may be stored at –20°C for up to 6 months.
4. Frozen plasma should be thawed rapidly at 37°C. Thawed plasmas should be stored at 2°-8°C and assayed within 4 hours.

PROCEDURE

Materials Provided – See Reagents

Materials Required But Not Provided

0.22 µm filtered deionized water
50-300 µL eight channel multi-pipette
0-20 µL, 20-200 µL, 200-1000 µL single pipettes
microwell plate reader for reading absorbance at 450 nm
microwell plate orbital shaker
microwell plate washer (optional)
0.5 M H_2SO_4 Caution: Handle sulfuric acid with great care. Avoid any skin and eye contact. Wear protection glasses and gloves when handling.

Preparing ADAMTS13 Standards

1. Open the foil pouch and remove the microwell strips/frame assembly. Remove the strips that will not be used, return them to the foil pouch and tightly reseal the pouch with the desiccant inside. Store the foil pouch at 2° - 8°C.
2. Reconstitute the ADAMTS13 Standard as instructed under REAGENT PREPARATION. Prepare five (5) serial dilutions of the standard using one of the following methods (Step 3a or 3b below). The concentrations of the serially diluted standard will be 100, 50, 25, 12.5, 6.25 or 3.12 ng/mL, respectively.

3a. Well Dilution Method: Add 200 µL of Standard to microwells A1/A2. Add 100 µL of Assay Buffer to microwells B1/B2 – F1/F2. Serially dilute the Standard by pipetting 100 µL of the standard from microwells A1/A2 into microwells B1/B2. Mix and pipette 100 µL from wells B1/B2 to wells C1/C2. Repeat this process through wells F1/F2. Remove and discard 100 µL from wells F1/F2. Add 100 µL of Assay Buffer to wells G1/G2 which serves as the 0 ng/mL standard.

3b. Microcentrifuge Tube Dilution Method. Pipette 225 µL Assay Buffer into 5 appropriate sized tubes, which are labeled 50, 25, 12.5, 6.25 or 3.12 ng/mL. Add 225 µL of Standard into the tube labeled 50 ng/mL and mix. Remove 225 µL of the diluted Standard from the 50 ng/mL tube and add this to 25 ng/mL tube and mix. Continue this process until all the tubes have diluted Standard. Transfer 100 µL of undiluted Standard to wells A1 and A2. Transfer 100 µL of the diluted Standard from the 50 ng/mL tube to wells B1 and B2. Similarly, transfer the remainder of the tubes with diluted Standard to the successive wells of the plate. Add 100 µL of Assay Buffer to wells G1 and G2, which serves as the 0 ng/mL standard.

Assay Procedure

4. Dilute each plasma sample 1:20 (1 part plasma + 19 parts Assay Buffer). Add 100 µL of Positive Control or diluted sample to a microwell, cover with the acetate sheet provided and incubate for 1 hour at 37°C.
5. Empty the contents of the microwells and wash 4 times with Wash Buffer. Washing may be performed using a microwell plate washer or manually. For manual washing, fill the wells with Wash Buffer with a pipette or squeeze bottle then empty and remove droplets by tapping the plate 4-5 times face down against absorbing material.
6. Add 100 µL of Detection Antibody to each microwell, cover with the acetate sheet and incubate for 30 minutes at room temperature (18-25°C) on an orbital microwell plate shaker with agitation (at 500 rpm).
7. Wash the wells by repeating Step 5.
8. Add 100 µL of working strength Enzyme Conjugate to each microwell, cover with the acetate sheet and incubate for 30 minutes at room temperature (18-25°C) on an orbital microwell plate shaker with agitation (at 500 rpm).
9. Wash the wells by repeating Step 5.
10. Add 100 µL of Substrate to each microwell immediately after the wash step, cover the wells with the acetate sheet and incubate for 5 minutes at room temperature (18°-25°C), during which time the substrate will turn blue.
11. Stop the enzymatic reaction by adding 50 µL of 0.5M H_2SO_4 to each microwell. Add the acid with the same speed and order as you added the substrate. Tap the sides of the microwell frame to ensure even distribution of the H_2SO_4. The solution color will turn yellow. Read the absorbances using microwell plate reader set to a wavelength of 450 nm within 10 minutes.

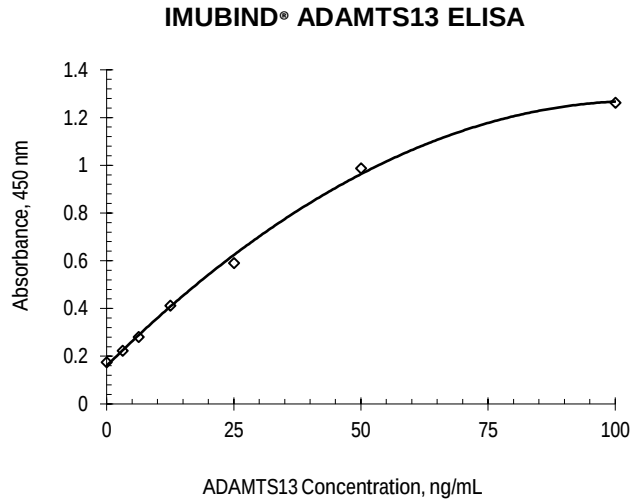
QUALITY CONTROL

The Positive Control plasma should be included each time the assay is performed.

RESULTS

Construct a standard curve by plotting the mean absorbance value for each standard versus its corresponding concentration of ADAMTS13. A standard curve should be generated each time the assay is performed. The following standard curve is for demonstration purposes only.

Representative Standard Curve



CALCULATION

Determine the amount of ADAMTS13 in the diluted plasma sample by interpolating directly from the standard curve. Since the unknown plasma sample is diluted 1:20 during preparation, multiply the results by 20 in order to obtain the concentration of ADAMTS13 in the neat plasma sample. The calculation is,

$$[\text{ADAMTS13}]_{\text{Plasma Sample}} = [\text{ADAMTS13}]_{\text{Diluted Test Sample}} \times 20$$

LIMITATIONS OF THE PROCEDURE

Platelet contamination in plasma samples will interfere with the assay results. Plasma samples must be free of platelets in order to have a valid result. Exercise great care to minimize disruption of the platelet pellet while recovering the platelet poor plasma. Samples should not be frozen and thawed more than two (2) times.

Samples should not be collected with EDTA as the anticoagulant. Icteric, lipemic and hemolyzed samples may interfere with the assay.

BIBLIOGRAPHY

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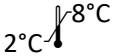
8. Starke, R., *et al.* *Brit J Haematol* 2006, 136: 649-655.

DEFINITION OF SYMBOLS

	Consult instructions for use
	Refer to SDS
	Manufactured by
	Store at 2°C to 8°C
LOT	Batch code / Lot number
	Expiration Date
REF	Catalog number
	Contains sufficient for <n> tests
CONT	Contains...