

IMUBIND® ADAMTS13/FXI Complex ELISA

Product No. 811

For Research use Only

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INTENDED USE

The IMUBIND® ADAMTS13/FXI Complex ELISA is intended for the measurement of ADAMTS13/FXI complexes in human plasma. The assay is limited 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) at the Tyr(1605)-Met(1606) bond located in the A2 region of vWF.¹ Studies have shown that low levels of ADAMTS13 activity are associated with Thrombotic Thrombocytopenia Purpura (TTP), a life-threatening hematological condition characterized by low platelet count, microvascular thrombi, red cell fragmentation, CNS and renal complications.^{2,3} A deficiency or low level of ADAMTS13 activity (<5%) may lead to an accumulation of UL-vWF multimers.⁴ The UL-vWF multimers will bind to receptors on platelets inducing platelet aggregation and formation of intravascular thrombi.

Congenital TTP is a rare inheritable disorder resulting from mutations in the ADAMTS13 gene. Genetic alterations have been identified at many sites within the ADAMTS13 gene and result in the production of non-functional ADAMTS13 protein.^{5,6} The acquired form of TTP is an autoimmune-like disorder caused by the development of autoantibodies to ADAMTS13 that inhibit its activity.⁷ Recent studies have identified patients with clinical symptoms of TTP that possess normal levels of ADAMTS13 and patients that possess low ADAMTS13 activity without symptoms of TTP.⁸ This suggests that other biological factors along with ADAMTS13 may play a role in TTP. Research has shown that ADAMTS13 forms stable complex with factor XI (FXI) and factor XIa (FXIa) and that these complexes are found in plasma.⁹ Measurements of ADAMTS13/FXI complexes in plasma may be useful towards the understanding of the role ADAMTS13 and FXI play in etiology and biology of congenital and/or acquired TTP.

PRINCIPLE OF THE METHOD

Diluted plasma samples are added to microwells coated with a polyclonal antibody reactive with a peptide in the C-terminal region of the ADAMTS13 molecule. During an incubation period, ADAMTS13 present in the sample will bind to the antibody coated to the wells. Following a washing step, a goat anti-FXI antibody labeled with horseradish peroxidase (HRP) is added to the microwells and binds to the ADAMTS13/FXI complexes captured on the plate during a short incubation period. 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 the 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/FXI complexes in the diluted plasma sample.

REAGENTS

96 antibody coated microwells (6 x 16), with acetate cover sheet
2 vials of Assay Buffer, 15 mL (lyophilized)
2 vials of Plasma Standard, 0.6 mL (lyophilized)
2 vials of Positive Control, 0.25 mL (lyophilized)

1 vial of Detection Antibody, goat anti-human FXI-HRP IgG, 120 µL
1 vial of Substrate, TMB (11 mL)
1 packet of Wash Buffer, PBS with 0.05% Tween 20, pH 7.4

WARNING

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 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.

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, protected from any moisture.
- 2. Assay Buffer:** Reconstitute a vial 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. Plasma Standard:** Reconstitute a vial with 0.6 mL of Assay Buffer. This standard corresponds to a normal plasma at a 1:4 dilution (25%). Plasma may be aliquoted and stored at -20°C for 6 months.
- 4. Positive Control:** Reconstitute a vial with 0.25 mL of Assay Buffer. This control corresponds to a plasma at a 1:10 dilution. The control may be aliquoted and stored at -20°C for 6 months.
- 5. Detection Antibody, Goat Anti-Human FXI-HRP:** Supplied as a concentrate, dilute the Detection Antibody 1:100 with Assay Buffer just prior to use. For running all 96 microwells at one time, dilute 100 µL of Detection Antibody up to 10 mL in Assay Buffer. If all 96 microwells are not to be used, dilute 20 µL of Detection Antibody up to 2 mL in Assay Buffer for each 16 microwell strip that will be used. Working strength Detection Antibody is stable for 2 hours at 2°-8°C. Discard any unused working strength Detection Antibody.
- 6. Substrate, TMB:** Supplied ready to use. Once opened, it may be used for up to 4 weeks when stored at 2°-8°C.
- 7. Wash Buffer:** Dissolve the contents of the packet with 900 mL 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. See "Collection, Transport and Processing of Blood Specimens for Testing Plasma-based Coagulation Assays; Approved Guidelines-Fourth Edition", NCCLS Document H21-A4, Vol. 23, No. 35, December 2003. 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.

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PROCEDURE

Materials Provided – See Reagents

Material Required But Not Provided

0.22 µm filtered deionized H₂O
50-300 µL eight channel multi-pipette
0-200 µL, 200-1000 µL single pipettes
microwell plate reader for reading absorbance at 450 nm
microwell plate washer (optional)
0.5 M H₂SO₄ **Caution:** Handle Sulphuric acid with great care. Avoid any skin and eye contact. Wear protection glasses and gloves when handling.

Preparing ADAMTS13/FXI 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 Plasma Standard as instructed under REAGENT PREPARATION. Prepare five (5) serial dilutions of the Plasma Standard as follows. Add 200 µL of Plasma Standard to microwells A1/A2. Add 100 µL of Assay Buffer to microwells B1/B2 – F1/F2.
3. Serially dilute the Plasma Standard by pipetting 100 µL of the plasma 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. The concentrations of the serially diluted Plasma Standard will be 25%, 12.5%, 6.25%, 3.125%, 1.56% and 0.78% respectively. Add 100 µL of Dilution Buffer to wells G1/G2 to serve as the 0% standard.

Assay Procedure

4. Dilute each plasma sample 1:10 (1 part plasma + 9 parts Assay Buffer). Add 100 µL of Positive Control or diluted sample to a microwell, cover with the acetate sheet and incubate for 2.5 hours at room temperature (18-25°C).
5. Empty the contents of the microwells and wash 4 times with Wash Buffer. Washing can be performed either using microwell plate washing equipment or manually (fill the wells with Wash Buffer with a pipette or squeeze bottle, wait three minutes, empty and remove droplets by tapping the plate 4-5 times face down against absorbing material).
6. Add 100 µL of working strength Detection Antibody to each well, cover with the acetate sheet and incubate the wells for 1 hour at room temperature (18-25°C) on an orbital microwell plate shaker with agitation (at 250 rpm).
7. Wash the wells by repeating Step 5.
8. 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). A blue color will develop.
9. Stop the enzymatic reaction by adding 50 µL of 0.5M H₂SO₄ 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 H₂SO₄. The solution color will turn yellow. . Read the absorbances on a microwell plate reader at a wavelength of 450 nm within 10 minutes.

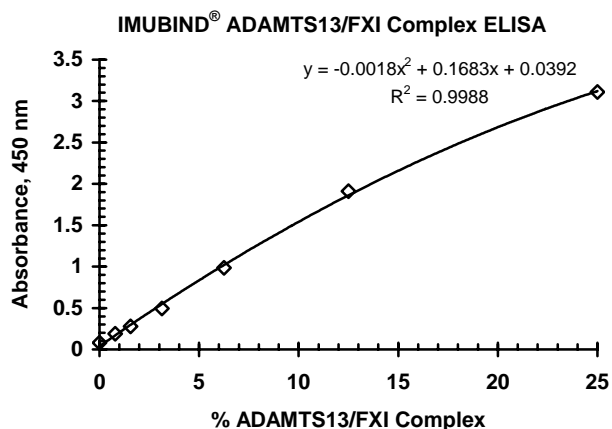
QUALITY CONTROL

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

RESULTS

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

Representative Standard Curve



CALCULATIONS

Determine the amount of ADAMTS13/FXI complexes in the diluted plasma sample by interpolating directly from the standard curve. As the plasma sample is diluted 1:10 during its preparation, multiply the results by 10 in order to obtain the concentration of ADAMTS13/FXI complexes in the neat plasma sample. The calculation is,

$$[\text{ADAMTS13/FXI}]_{\text{Plasma Sample}} = [\text{ADAMTS13/FXI}]_{\text{Diluted Test Sample}} \times 10$$

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 in minimizing disruption of the platelet pellet while recovering the platelet poor plasma. Samples should not be frozen and thawed more than two times.

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

EXPECTED VALUES

The level of ADAMTS13/FXI complex in acquired TTP plasmas is reduced relative to normal plasma.⁹

PERFORMANCE CHARACTERISTICS

Precision

The intra-assay coefficient of variation (CV) for this ELISA has been found to be 5.6%.

Specificity

The capture antibody is highly specific for ADAMTS13 and the detection antibody is highly specific for human FXI.

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