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IMUBIND® FSAP ELISA

Product no. ADG876

Storage: 2 – 8°C **For Research Use Only!**

PRODUCT INSERT ENGLISH

INTENDED USE

The IMUBIND® FSAP ELISA is intended for the measurement of factor seven activating protease in human plasma. The assay is intended for research use only.

EXPLANATION OF THE TEST

Factor Seven Activating Protease (FSAP, Factor VII activating protease, FVII activating protease) is a potent activator of factor VII independent of tissue factor and an activator of pro-urokinase. Present in human plasma at a concentration of 12 µg/mL (1 PEU/mL)[1], FSAP exists as a single-chain proenzyme with a molecular ratio of 64 kDa. The proenzyme can be activated by an autocatalytic mechanism or by urokinase generating an active two-chain form consisting of a 40 kDa heavy chain and a 30 kDa light chain which possesses the protease domain. The autoactivation is enhanced in the presence of heparin. Conversely, calcium ions stabilize single-chain FSAP and retards the autoactivation.[2] FSAP playing a dual role, clot formation via factor VII activation and clot degradation via pro-urokinase activation, holds a key position in the delicate balance of the hemostatic system.

A mutant variant of FSAP with a single nucleotide polymorphism (SNP) has been identified, termed "Marburg I" (FSAP MI). The FSAP Marburg I variant shows a reduced ability to activate pro-urokinase, whereas its ability to activate Factor VII is normal.[3] It seems likely that FSAP Marburg I, due to the resulting haemostatic imbalance, may promote the development of thromboembolic diseases. The FSAP Marburg I variant was found to have an effect upon the risk for cardiovascular heart disease in those patients with elevated levels of cholesterol and triglyceride.[4] In addition, the FSAP Marburg I variant was found to be a significant risk predictor for the evolution and progression of carotid stenosis[5] and associated with idiopathic venous thromboembolism.[6]

PRINCIPLE OF THE METHOD

Diluted plasma samples are added to microwells coated with a monoclonal antibody directed against the light-chain of human FSAP. During an incubation period, FSAP present in the sample will bind to the antibody coated to the wells. Following a washing step, a streptavidin-horseradish peroxidase (SA-HRP) conjugated monoclonal antibody directed against the Glu^{360} residue on the light-chain is added to the microwells and binds to FSAP captured on the plate. Following another washing step, the addition of a perborate-3,3'-5,5'-tetramethylbenzidine (TMB) substrate and its subsequent reaction with the HRP present creates a blue colored solution. The enzymatic reaction is stopped by adding citrate stop solution, 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 FSAP in the diluted plasma sample.

REAGENTS

- MTP **Antibody Coated Microtiter plate,** MTP-96 (12x8) well
- WASH **Wash buffer**, 50 ml, 1 vial (concentrate)
- DILB **Dilution buffer**, 50 ml, 1 vial (ready-to-use)
- STD **Standard,** 500 µl human plasma, 1 vial (lyophilized)
- AB **Antibody conjugate**, HRP-conjugated anti-human FSAP, 120 µl, 1 vial (100x concentrate)
- TMB **TMB Substrate**, 12 ml, 1 vial (ready-to-use)
- STOP **Stop solution**, 6 ml, 1 vial (ready-to-use)

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 origin 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.

Not for internal use in humans or animals. Do not use the kit components beyond the expiration date. Do not mix reagents from different kit lots. 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 or ingest reagents. Wear laboratory coat and disposable gloves throughout the test procedure and wash hands thoroughly afterwards. Handle gently; avoid splashing, foam, or aerosol formation.

WASH DILB AB	Warning		H317, P280, P333+P313
STOP	Warning		H314, P303+P361+P353, P305+P351+P338, P310

Hazard Statements:
H317 May cause an allergic skin reaction.
H314 Causes severe skin burns and eye damage

Precautionary Statements:
P280 Wear protective gloves/ protective clothing/ eye protection/ face protection
P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention.
P303 + P361 + P353 IF ON SKIN (or hair): Remove/ Take off immediately all contaminated clothing. Rinse skin with water/shower.
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 CENTRE or doctor/physician.

REAGENT PREPARATION AND STORAGE

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

MTP **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.

WASH **Wash buffer**: If crystals are visible, incubate the vial in a 37°C water bath a few minutes until the crystals are dissolved. Transfer the content to a 1 liter bottle and fill up the concentrate to 1 liter with filtered deionized/distilled water. Diluted Wash Buffer may be used for up to 4 weeks when stored at 2°-8°C.

DILB **Dilution buffer:** Supplied ready to use. Opened dilution buffer is stable for 3 month when stored at 2°-8°C.

STD **FSAP Plasma Standard**: Reconstitute a vial with 0.5 mL of filtered deionized or distilled water. Plasma may be aliquoted and stored at -20°C for 6 months.

AB **Antibody conjugate:** Supplied as a concentrate, dilute the Antibody conjugate 1:100 with Dilution buffer just prior to use. For using all 96 microwells at one time, dilute 100 µL of Antibody conjugate to 10 mL in Dilution Buffer. If not all 96 microwells are used, dilute 10 µL of Antibody conjugate to 1 mL in Dilution buffer for each 8-microwell strip that will be used. Working strength Antibody conjugate is stable for 4 hours at 2°-8°C. Discard any unused working strength

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Antibody conjugate. Opened antibody is stable for 3 month when stored in the dark at 2°-8°C.

TMB **Substrate, TMB:** Supplied ready to use. Opened substrate is stable for 3 month when stored in the dark at 2° - 8°C.

STOP **Stop solution:** Supplied ready to use. Opened stop solution is stable for 3 month 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 or heparin. 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 5,000 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

Material Required But Not Provided

0.22 µm filtered deionized H_2O
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), microwell plate shaker (optional)

Preparing FSAP Standards

1. Reconstitute the FSAP Plasma Standard as instructed under REAGENT PREPARATION. In a plastic test tube, dilute the standard 1:10 with Dilution Buffer. This diluted plasma serves as the highest Standard (1) in the ELISA.

2. Make serially dilutions of the (1) FSAP Standard with Dilution Buffer as follows. Use Dilution Buffer as the 0% standard.

Tube		Dilution buffer	Conc. [mPEU/ml]
(1)	50 µl reconst. plasma	450 µl	2
(2)	250 µl from (1)	250 µl	1
(3)	250 µl from (2)	250 µl	0.5
(4)	250 µl from (3)	250 µl	0.25
(5)	250 µl from (4)	250 µl	0.125
(6)	250 µl from (5)	250 µl	0.0625
(7)	-	250 µl	0

Preparing the Sample Dilutions

3. Dilute each plasma sample 1:1000 with dilution buffer. It is recommended to make a 1:10 pre-dilution (5 µl plasma + 45 µl dilution buffer) and then a 1:100 dilution (5 µl pre-diluted plasma + 495 µl dilution buffer).

Running standard and samples in duplicate is recommended.

Assay Procedure

4. 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.

5. Add 100 µL of FSAP Standard or diluted sample to a microwell, cover with the acetate sheet and incubate at room temperature (18-25°C) for 1 hour on an orbital microwell plate shaker with agitation (at 250 rpm).

6. Empty the contents of the microwells and wash 4 times with Wash Buffer. Washing may 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).

7. Add 100 µL working strength Antibody conjugate to each microwell, cover with the acetate sheet and incubate the wells at room temperature (18-25°C) for 30 minutes on an orbital microwell plate shaker with agitation (at 250 rpm).

8. Wash the wells by repeating Step 6.

9. Add 100 µL of Substrate to each microwell immediately after the wash step, cover the wells with the acetate sheet and incubate for 5-10 minutes at room temperature (18°-25°C). A blue color will develop.

10. Stop the enzymatic reaction by adding 50 µL of Stop solution to each microwell. Add the Stop solution with the same speed and order as you added the substrate. Tap the sides of the microwell frame to ensure even distribution of the Stop solution. The solution color will turn yellow. Read the absorbances on a microwell plate reader at a wavelength of 450 nm within 10 minutes.

RESULTS

Construct a standard curve by plotting the mean absorbance value for each FSAP standard versus its corresponding concentration. A standard curve should be generated each time the assay is performed. The following standard curve is for demonstration purposes only. Data are expressed as mPEU/ml (PEU: Plasma Equivalent Units).

CALCULATIONS

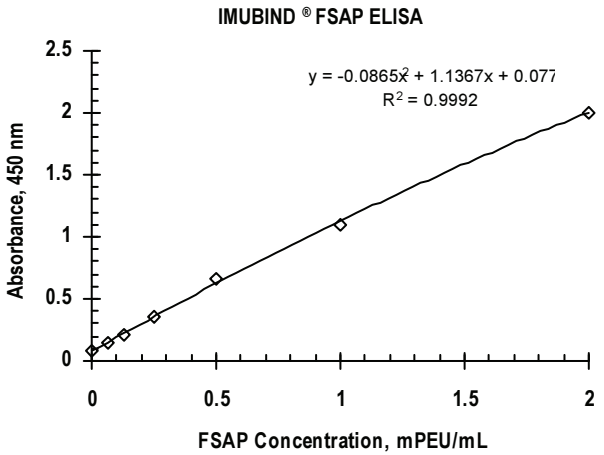
Determine the amount of FSAP in the diluted plasma sample by interpolating directly from the standard curve. As the plasma sample was diluted 1:1000 during its preparation, multiply the results by 1000 in order to obtain the concentration of FSAP in the neat plasma sample. The calculation is:

$$[FSAP]_{Plasma\ Sample} = [FSAP]_{Diluted\ Test\ Sample} \times 1000$$

LIMITATIONS OF THE PROCEDURE

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

Representative Standard Curve



EXPECTED VALUES

The level of FSAP in citrated pooled normal plasma was estimated to be 12.0 µg/mL.[1] In a study of 189 healthy individuals, the mean FSAP level was found to be slightly less than 12.0 µg/mL, with women having slightly higher levels than men (11.15 µg/mL to 10.51 µg/mL). There was no apparent difference depending upon the age of the individual.[7]

In a study of samples from individuals with a variety of pathological conditions (stroke, thrombophilia, DVT, PE, MI and pregnancy), the mean FSAP level was found to range from approximately 7 µg/mL for stroke patients up to 9.6 µg/mL for pregnant women.[8]

Each laboratory should establish its own normal range using the local population.

PERFORMANCE CHARACTERISTICS

Precision

The intra- and inter-assay coefficients of variations (CV) for this ELISA have been estimated to be 3.8% and 9.7% respectively.

Specificity

The capture and detection antibodies are highly specific for human FSAP.

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8. Data on File, Sekisui Diagnostics Inc.

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