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IMUBIND® FSAP Marburg I ELISA

Product No. 878

Storage: 2–8°C

For Research Use Only!

INTENDED USE

The IMUBIND® FSAP Marburg I ELISA is intended for the detection of the Marburg I (MI) variant of factor seven activating protease (FSAP) 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. FSAP playing a dual role, clot formation via factor VII activation and clot degradation via prourokinase 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 prevalence of the heterozygous FSAP MI genotype (1601 G/A) has been reported to be in a range of 4 to 9 % in Caucasian populations. The FSAP Marburg I variant shows a reduced ability to activate prourokinase, whereas its ability to activate Factor VII is normal.³ It seems likely that FSAP Marburg I, due to the resulting hemostatic 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 stenosis⁵ 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 Marburg I variant of human FSAP. During an incubation period, FSAP Marburg I 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 FSAP 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

- R1** Antibody Coated Microtiter plate, MTP-96 (12x8) well
- R2** Wash buffer, 50 ml, 1 vial (concentrate)
- R3** Dilution buffer, 50 ml, 1 vial (ready-to-use)
- R4** FSAP wild type control, 0.5 ml human plasma, 2 vials (lyophilized)
- R5** FSAP Marburg I control, 0.5 ml human plasma, 2 vials (lyophilized)
- R6** Antibody conjugate, HRP-conjugated anti-human FSAP, 120 µl, 1 vial (concentrate)
- R7** Substrate, 11 ml, 1 vial (ready-to-use)
- R8** 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.

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.

REAGENT PREPARATION AND STORAGE

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

- R1 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.
- R2 Wash buffer:** 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.
- R3 Dilution buffer:** Supplied ready to use. Opened dilution buffer is stable for 3 month when stored at 2°-8°C.
- R4 FSAP wild type control:** 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.
- R5 FSAP Marburg I control:** 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.
- R6 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 Antibody conjugate. Opened antibody is stable for 3 month when stored in the dark at 2°-8°C.
- R7 Substrate, TMB:** Supplied ready to use. Opened substrate is stable for 3 month when stored in the dark at 2° - 8°C.
- R8 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. 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.

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), microwell plate shaker (optional)

Preparing FSAP Controls

1. Reconstitute the FSAP controls as instructed under REAGENT PREPARATION. The resuspended plasma controls are used without further dilution.

Preparing the Sample Dilutions

2. Dilute each plasma sample 1:100 with dilution buffer (e.g. 2 µl plasma + 198 µl dilution buffer)

Running controls and samples in duplicate is recommended.

Assay Procedure

3. 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.
4. Add 100 µL of FSAP wild type control, FSAP Marburg I control 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).
5. 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).
6. 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).
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 15 minutes at room temperature (18°-25°C). A blue color will develop.
9. Stop the enzymatic reaction by adding 50 µL of Stop solution 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 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.

LIMITATIONS OF THE PROCEDURE

Samples should not be collected with EDTA as the anticoagulant. Samples containing high mol wt (>10 kDa) dextran sulfate will lead to false positive results. Icteric, lipemic, hemolyzed, and RF/HAMA-positive samples may interfere with the assay.

If the wash steps are not correctly performed, the negative control can produce a high absorbance value. In order to avoid non-specific color development, check that the wash steps are performed efficiently.

RESULTS

Positive and negative controls provided in the kit allow validating the assay. Expected A450 values for positive and negative controls can present variations from lot to lot but they always are:

Positive Control A450 > 1.0
Negative Control A450 < 0.25

The positive/negative cut-off is defined as a A450 value of 0.5 x Positive Control A450.

PERFORMANCE CHARACTERISTICS

Precision

The intra- and inter-assay coefficients of variations (CV) for this ELISA have been estimated to be 3.3% and 11.7% respectively.

Specificity

The capture antibody is highly specific for the human FSAP MI variant.

BIBLIOGRAPHY

1. Römisch, J., Vermöhlen, S., Feussner, A. and Stöhr, H. A. The FVII activating protease cleaves single-chain plasminogen activators. *Haemostasis* 1999, **29**: 292-299.
2. Kannemeier, C., Feussner, A., Stöhr, H. A., Weisse, J., Preissner, K. T. and Roemisch, J. Factor VII and single-chain plasminogen activator-activating protease: activation and autoactivation of the proenzyme. *European Journal of Biochemistry* 2001, **268**(13): 3789-3796.
3. Roemisch, J., Feussner, A., Nerlich, C., Stöhr, H. A. and Weimer, T. The frequent Marburg I polymorphism impairs the pro-urokinase activating potency of the factor VII-activating protease (FSAP). *Blood Coagulation and Fibrinolysis* 2002, **13**: 433-441.
4. Ireland, H., Miller, G. J., Webb, K. E., Cooper, J. A. and Humphries, S. E. The factor VII activating protease G511E (Marburg) variant and cardiovascular risk. *Thrombosis and Haemostasis* 2004, **92**: 986-992.
5. Willeit, J., Kiechl, S., Weimer, T., Mair, A., Santer, P., Wiedermann, C. J. and Roemisch, J. Marburg I polymorphism of factor VII-activating protease: a prominent risk factor of carotid stenosis. *Circulation* 2003, **107**: 667-670.
6. Hoppe, B., Tolou, F., Radtke, H., Kiesewetter, H., Dorner, T. and Salama, A. *Blood* 2005, **105**: 1549-1551.
7. Römisch, J., Feussner, A. and Stöhr, H. A. Quantitation of the factor VII- and single-chain plasminogen activator-activating protease in plasma of healthy subjects. *Blood Coagulation and Fibrinolysis* 2001, **12**: 375-383.
8. Data on File, American Diagnostica Inc.