

IMUCLONE® FPA ELISA

Product No. 635

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INTENDED USE

The IMUCLONE FPA ELISA is a competitive enzyme-linked immunosorbent assay (CELIA) for measuring human FPA on bentonite adsorbed human plasma or in any fluid where FPA may be present. The assay is limited to "Research Use Only" in the United States.

EXPLANATION OF THE TEST

FPA, Fibrinopeptide A, is a 1536 D molecular ratio, 16 amino acid peptide released from the amino terminus of fibrinogen A α chains by thrombin cleavage. Two molecules of FPA are released per molecule of fibrinogen. Elevated blood levels of FPA are indicative of excess thrombin activity, found in various clinical states such as DIC and evolutive thrombosis.

PRINCIPLE OF THE PROCEDURE

Following the removal of any cross-reactive fibrinogen by bentonite adsorption, diluted plasma samples, biological fluid or FPA Calibrator are preincubated with an affinity purified rabbit anti-human FPA polyclonal antibody. Then the test solutions are added to microwells precoated with FPA. Anti-human FPA antibodies that did not bind to FPA during the preincubation step will bind to the FPA coated to the microwells. Following a wash step, a goat anti-rabbit IgG polyclonal antibody coupled to horseradish peroxidase (HRP) is added to the microwells and binds to the immobilized FPA antibodies. Following another wash step, the peroxidase substrate 3,3',5,5'-tetramethylbenzidine (TMB), in the presence of hydrogen peroxide, is added to the microwells and the subsequent reaction yields a blue colored solution. Addition of sulfuric acid stops the reaction and turns the solution color yellow. The absorbance of the solution is measured at 450 nm. The absorbance is indirectly proportional to the amount of FPA present in the tested sample.

REAGENTS

- 1 vial of bentonite suspension, ready to use (50 mL)
- 1 vial of 2% Tween 20, ready to use (5 mL)
- 12 strips of human FPA coated microwells, 8 wells per strip in holder
- 1 vial of Sample Diluent, ready to use (50 mL)
- 3 vials of FPA Calibrator, 50 ng/mL (lyophilized)
- 3 vials of Rabbit Anti-Human FPA (lyophilized)
- 3 vials of Anti-Rabbit IgG-HRP Immunoconjugate (lyophilized)
- 1 vial of Conjugate Diluent, ready to use (25 mL).
- 1 vial Wash Solution, 20 fold concentrate (50 mL)
- 1 vial of Substrate, TMB containing hydrogen peroxide (25 mL)
- 1 vial of Special Anticoagulant Solution, ready to use (20 mL)
- 1 vial of 0.45M Sulfuric Acid, ready to use (6 mL)

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 (OPD is toxic). Avoid splashing or aerosol formation.

REAGENT PREPARATION AND STORAGE

Unopened and lyophilized reagents are stable until the expiration date printed on the box when properly stored at 2°-8°C.

1. Bentonite Suspension: **Supplied ready to use. Mix thoroughly before use. Once opened, it may be used for up to 4 weeks when stored at 2°-8°C. This diluent contains 0.09% sodium azide as a preservative.**
2. 2% Tween 20: **Supplied ready to use. Once opened, it may be used for up to 4 weeks when stored at 2°-8°C. This diluent contains 0.09% sodium azide as a preservative.**
3. FPA Coated Microwell Strips: **Once removed from the aluminium 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, and stored in the provided storage bag.**
4. Sample Diluent: **Supplied ready to use, once opened, the diluent may be used for up to 4 weeks when stored at 2°-8°C. This diluent contains 0.05% Kathon CG as a preservative.**
5. FPA Calibrator: **Reconstitute each vial with 2 mL of Sample Diluent to generate the 50 ng/mL FPA calibrator. The calibrator is stable for at least 8 hours at room temperature.**
6. Rabbit Anti-Human FPA: **Reconstitute each vial with 2 mL of Sample Diluent. The antibody is stable for 1 week when stored at 2°-8°C.**
7. Conjugate Diluent: **Supplied ready to use. Once opened, it may be used for up to 4 weeks when stored at 2°-8°C. This diluent contains 0.05% Kathon CG as a preservative.**
8. Anti-Rabbit IgG-HRP Immunoconjugate: **Reconstitute each vial with 7.5 mL of Conjugate Diluent. Shake the vial gently to homogenize the content. Reconstituted immunoconjugate is stable for at least 24 hours at room temperature or for at least 7 days at 2°-8°C.**
9. Wash Solution: **If solids are present, incubate the vial for 15-30 minutes in a 37°C water bath. Shake the vial and dilute the amount required 1:20 in distilled water (the entire vial is sufficient to prepare 1 Liter of Wash Solution). The Wash Solution may be used for up to 4 weeks after opening when stored at 2°-8°C in its original vial. Diluted Wash Solution may be used for up to 7 days when stored at 2°-8°C.**
10. TMB Substrate: **Supplied ready to use. Once opened, it may be used for up to 4 weeks when stored at 2°-8°C.**
11. Special FPA Anticoagulant Solution: **Supplied ready to use. Once opened, it may be used for up to 4 weeks when stored at 2°-8°C.**
12. 0.45M Sulphuric Acid: **Supplied ready to use.**

SPECIMEN COLLECTION AND PREPARATION

Platelet poor plasma specially collected with an anticoagulant for FPA must be used for this assay. Plasma collection should be performed as follows:

1. **Collect 9 parts of blood into 1 part of the Special Anticoagulant Solution provided in the assay (contains Trisodium Citrate, Heparin, Hirudin, Aprotinin and sodium azide). The first few drops of blood should be discarded.**
2. **Centrifuge the blood sample at 2,500 rpm for 20 minutes.**
3. **Plasma must be treated with bentonite within 8 hours after collection or stored at -20°C within 4 hours after collection. Plasma may be stored at -20°C for up to 1 month.**
4. **Frozen plasma should be thawed rapidly at 37°C just before use and treated with bentonite.**

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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)

Bentonite Treatment

Cross-reactive fibrinogen must be removed by bentonite adsorption. Thoroughly mix bentonite suspension in order to make it homogeneous. Add 0.5 mL of the bentonite solution to 1.0 mL of the anticoagulated plasma. Mix for 10 minutes (using an end-over-end agitator if available). Centrifuge the mixture for 20 minutes at 2,500 g and collect 1.0 mL of supernatant.

Repeat the process by adding again 0.5 mL of bentonite suspension to the 1.0 mL of supernatant, mix and centrifuge. The bentonite treated plasma is then fibrinogen free. It must be used within:

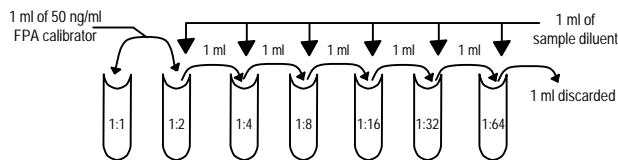
- 24 hours stored at room temperature or at 2-8°C.
- 1 month when stored frozen at -20°C or below.

Just before use, add 50 µL of 2% Tween 20 to 1.0 mL of bentonite adsorbed plasma.

Preincubation of Samples and Standards

To 1.0 mL of each bentonite-adsorbed plasma/Tween 20 sample, add 0.1 mL of Rabbit Anti-Human FPA IgG and incubate for 1 hour at 37°C.

As depicted below, prepare serial dilutions of 1:1 down to 1:64 of the 50 ng/mL FPA Calibrator in the Sample Diluent.



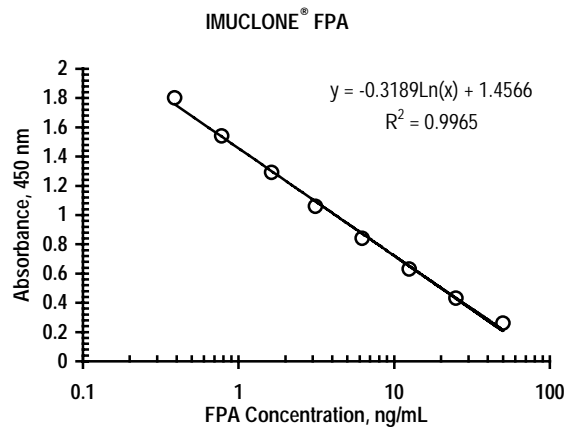
FPA Standards ranging from 0.78 ng/mL up to 50 ng/mL are created. Add 0.1 mL of Rabbit Anti-Human FPA to each 1.0 mL of FPA Standard. Incubate for 1 hour at 37°C.

Remove the required number of strips from the aluminium pouch, for the series of measures to be performed. Place the strips in the frame provided. Add the reagents into the appropriate microwells as indicated on the following table:

Reagent	Volume	Procedure
FPA Standard, test sample or Sample Diluent (blank)	200 µL	Add the standard or test samples to an appropriate microwell.
Incubate for 1 hour at room temperature (18°-25°C).		
Wash Solution	300 µL	Wash the wells 5 times.
Anti-Rabbit IgG-HRP Conjugate	200 µL	Add the conjugate to each microwell.
Incubate for 1 hour at room temperature (18°-25°C).		
Wash Solution	300 µL	Wash the wells 5 times.
TMB Substrate	200 µL	Add the substrate Immediately after washing the wells.
Incubate for exactly 5 minutes at room temperature (18-25°C).		
0.45M H ₂ SO ₄	50 µL	Following exactly the same time intervals used for adding the substrate, stop the reaction by adding 0.45M H ₂ SO ₄ .
Wait for 10 minutes to allow the color to stabilize and measure the absorbance at 450 nm.		

RESULTS

Construct a standard curve (linear vs. log) by plotting the mean absorbance value for each FPA standard on the ordinate versus the corresponding concentration of FPA on the abscissa. A standard curve should be generated each time the assay is performed. The following standard curve is for demonstration purposes only.



CALCULATIONS

From the standard curve obtained, directly interpolate the FPA concentration in the samples tested. The protocol for the bentonite treatment dilutes the plasma 1:2. Therefore, multiply the calculated FPA concentration by 2 to determine the FPA concentration in the original plasma sample. Alternatively, an ELISA software (i.e. Dynex, etc.) can be used for the calculation of concentrations.

LIMITATIONS OF THE PROCEDURE

There are no known limitations for the assay.

EXPECTED VALUES

The FPA concentration in normal human plasma is usually below 3 ng/mL.

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