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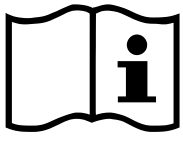
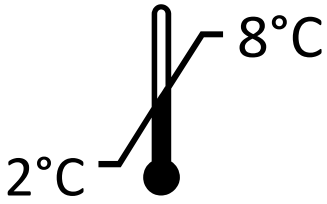
IMUCLONE™ FPA ELISA

REF 635

For Research Use Only





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.

For Research Use Only — Not for use in diagnostic procedures.

EXPLANATION OF THE TEST

FPA, Fibrinopeptide A, is a 1536 D molecular weight, 16 amino acid peptide released from the amino terminus of fibrinogen A alpha 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.

PRINCIPLE OF THE METHOD

Diluted plasma sample, following the removal of any cross-reactive fibrinogen by bentonite adsorption, FPA Calibrator or biological fluid are preincubated with an affinity purified rabbit anti-human FPA polyclonal antibody. Solutions are then added to microwells precoated with FPA. Anti-human FPA antibodies that did not bind to FPA during the preincubation step bind to the FPA coated microwells. Following a wash step, a goat anti-rabbit IgG polyclonal antibody-horseradish peroxidase (HRP) conjugate 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 solution absorbance 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)
96 Microtest Plate pre-coated with human FibrinoPeptide A; 12 strips of 8 wells in a frame
1 vial of Sample Diluent, ready to use (50 mL)
3 vials of FPA Calibrator, (lyophilized)
3 vials of Rabbit Anti-human FPA antibodies (lyophilized)
3 vials of Anti-Rabbit IgG HRP IC (Immunoconjugate) (lyophilized)
1 vial of Conjugate Diluent, ready to use (25 mL)
1 vial Wash Solution, 20 fold concentrate (50 mL)
1 vial of TMB Substrate (Peroxidase Substrate) (25 mL)
1 vial of Special Anticoagulant solution, ready to use (20 mL)
1 vial of Stop Solution (0.45 M Sulfuric Acid), ready to use (6 mL)

WARNINGS AND PRECAUTIONS

Source material for some of the reagents of this assay 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). 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.

For Research 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.

Conjugate Diluent	Warning 	CONT: Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1) H317, P261, P272, P280, P302+P352, P333+P313, P363, P501
Sample Diluent	Warning 	CONT: Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1) H317, P261, P272, P280, P302+P352, P333+P313, P363, P501
Wash Solution	Warning 	CONT: Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1) H317, P261, P272, P280, P302+P352, P333+P313, P363, P501
Stop Solution	Danger 	CONT: Sulfuric acid H290, P234, P390

Hazard Statements:	H290	May be corrosive to metals.
	H317	May cause an allergic skin reaction.
Precautionary Statements:	P234	Keep only in original container.
	P261	Avoid breathing dust/fume/gas/mist/vapors/spray.
	P272	Contaminated work clothing should not be allowed out of the workplace.
	P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
	P302 + P352	IF ON SKIN: Wash with plenty of soap water.
	P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
	P363	Wash contaminated clothing before reuse.
	P390	Absorb spillage to prevent material damage.
	P501	Dispose of contents/container in consultation with your regional waste disposer

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. Allow the reagents to warm to room temperature for 30 minutes before use. Warning: Bentonite Suspension, Tween 20 and Special Anticoagulant Solution contain sodium azide which may react with lead and copper tubing to form explosive metal azides. Flush waste solutions with large volumes of water when disposing down a sink.

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. **Microtest Strips pre-coated with human FibrinoPeptide A**: 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 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 FPA calibrator of concentration “C” ng/mL. See the enclosed flyer for the exact concentration. The calibrator is stable for at least 8 hours at room temperature.
6. **Rabbit Anti-human FPA antibodies**: 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 IC**: 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 4 weeks 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. This reagent contains 0.05% Kathon CG.
10. **TMB Substrate** (Peroxidase Substrate): Supplied ready to use. Once opened, it may be used for up to 4 weeks when stored at 2°-8°C.
11. **Special Anticoagulant solution**: Supplied ready to use. Once opened, it may be used for up to 4 weeks when stored at 2°-8°C. This reagent contains 0.09% sodium azide as a preservative.
12. **Stop Solution (0.45 M Sulfuric Acid)**: Supplied ready to use. Caution: Sulfuric acid is caustic. Handle with care. Avoid any skin and eye contact. Wear protective glasses and gloves when handling.

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). Discard the first few drops of blood.
2. Centrifuge the blood sample at 2,500 x g for 20 minutes.
3. Plasma must be treated with bentonite within 8 hours after collection or stored frozen at -20°C or below within 4 hours after collection. Plasma may be stored at -20°C or below for up to 1 month.
4. Frozen plasma should be thawed rapidly at 37°C just before use and treated with bentonite.

PROCEDURE

Materials Provided – See Reagents

Materials Required But Not Provided

Distilled water, 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 of Human Plasma Samples

Cross-reactive fibrinogen must be removed by bentonite adsorption. Thoroughly mix the Bentonite Suspension in order to make it homogeneous. Add 0.5 mL of the Bentonite Suspension 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 x 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:

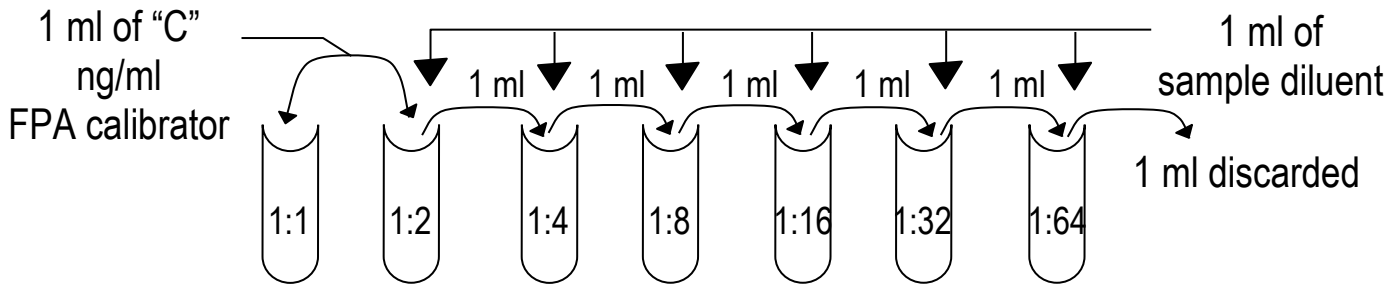
24 hours when held at room temperature or at 2-8°C.
1 month when stored frozen at –20°C or below.

Just before use, add 50 µL of 2% Tween 20 to 1.0 mL of bentonite-treated plasma. The bentonite-treated plasma is diluted 1:2 and the measured FPA concentration must be multiplied by 2 when calculating the results.

Preincubation of Samples and Standards

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

As depicted below, prepare serial dilutions of 1:1 to 1:64 of the FPA Calibrator at “C” ng/mL in the Sample Diluent to create the FPA standards.



FPA standards ranging from C ng/mL to C/64 ng/mL are created. Add 0.1 mL of Rabbit Anti-human FPA antibodies to each 1.0 mL of FPA standard. Incubate for 1 hour at 37°C.

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

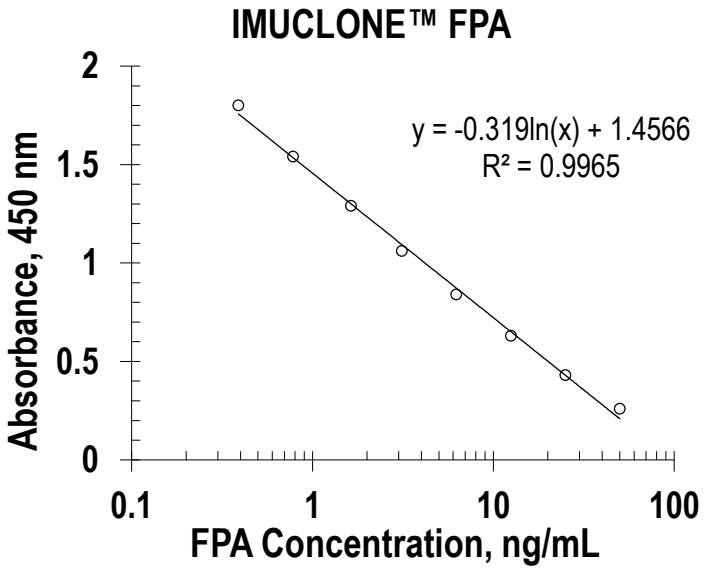
Reagent	Volume	Procedure
FPA standards, test sample or Sample Diluent (blank)	200 µL	Add the standard or test samples to an appropriate microwell.
Incubate for 1 hour at room temperature (18°-25°C).		
Wash Solution	300 µL	Wash the wells 5 times.
Anti-Rabbit IgG HRP IC	200 µL	Add the IC (immunoconjugate) 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).		
Stop Solution (0.45 M H_2SO_4)	50 µL	Following exactly the same time intervals used for adding the substrate, stop the reaction by adding 0.45 M H_2SO_4.
Wait for 10 minutes to allow the color to stabilize and measure the absorbance at 450 nm.		

Notes:

1. Avoid letting the plate in bright sunlight during incubations and particularly during color development.
2. Do not allow the microwells to dry out between the addition of reagents or following a washing step. Add the next reagent within 3 minutes to prevent the microwells from drying, which could damage the immobilized components. If necessary, fill the microwells with prepared Wash Solution and empty them just before the introduction of the next reagent.
3. When adding the TMB Substrate, the time interval between each row must be accurate and exactly determined. It must be the same when stopping the reaction.
4. For bichromatic absorbance readings, a reference wavelength of 690 nm or 620 nm may be used.

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.

EXPECTED VALUES

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

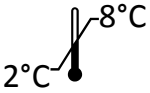
LIMITATIONS OF THE PROCEDURE

Human plasma must be pre-treated with bentonite to remove any fibrinogen present as it will react to the anti-FPA antibodies.

BIBLIOGRAPHY

1. Nossel, H. L., Younger, L. R., Wilner, G. D., Procupez, T., Canfield, R. E. and Butler, V. P. Jr. Radio-immunoassay of human fibrinopeptide A. *Proc Nat Acad Sci USA* 1971, **68**: 2350-53.
2. Soria, J., Soria, C. and Ryckewaert, J. J. A solid phase immuno enzymological assay for the measurement of human fibrinopeptide A. *Thromb Res* 1980, **20**: 425-435.
3. Amiral, J., Walenga, J. M. and Fareed, J. Development and performance characteristics of a competitive enzyme immunoassay for fibrinopeptide A. *Semin Thromb Hemost* 1984, **10**: 228-242.

DEFINITION OF SYMBOLS

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