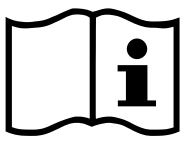
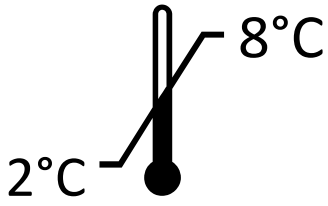
コスモ・バイオ株式会社
BioMedica Diagnostics
P.O. Box 1030, 94 Wentworth Road
Windsor, NS, Canada B0N 2T0
Tel: 1.902.798.5105 | Fax: 1.902.798.1025
info@biomedicadiagnostics.com | www.biomedicadiagnostics.com

IMUCLONE™ Platelet Factor 4 ELISA

REF 634

For Research Use Only





INTENDED USE

The IMUCLONE™ Platelet Factor 4 ELISA is an enzyme-linked immunosorbent assay for measuring human Platelet Factor 4 in platelet depleted plasma, or in any biological fluid where Platelet Factor 4 may be present.

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

EXPLANATION OF THE TEST

Platelet Factor 4 (PF4) is a 70 amino acid (Mr 7800 D) peptide monomer. PF4 is released from activated platelet α-granules in a tetrameric form complexed with platelet proteoglycan[1,3,4]. Upon release, the half-life of PF4 is very short, less than 5 minutes, as it quickly binds to endothelial cell glycosaminoglycans where it is stored. PF4 possesses a powerful anti-heparin activity as it binds to heparin, forming a stochiometric complex, where 1 mg of PF4 will inhibit 27 IU of heparin[2].

PRINCIPLE OF THE METHOD

Diluted plasma samples, biological fluid or PF4 standards are added to microwells precoated with an affinity purified rabbit polyclonal antibody specific for human PF4. The antibody captures the PF4 protein present in the solutions during an incubation period. Following a wash step, an affinity purified rabbit polyclonal antibody specific for human PF4 coupled to horseradish peroxidase (HRP) is added to the microwells and binds to the immobilized PF4.

Following another wash step, the peroxidase substrate 3,3',5,5' – tetramethylbenzidine (TMB), in presence of hydrogen peroxide (H_2O_2), is added to the microwell and the subsequent enzymatic reaction yields a blue colored solution. Last, the addition of sulfuric acid stops the reaction and turns the solution color to yellow. The absorbance of the solution is measured at 450 nm. The absorbance is directly proportional to the amount of PF4 present in the tested sample.

REAGENTS

96 Microwell Plate pre-coated with anti-(h)-PF4; 12 strips of 8 wells/strip in a frame holder plus storage bag with desiccant
2 vials of PF4 Sample Diluent, ready to use (50 mL)
3 vials of PF4 Standard (lyophilized)
1 vial of PF4 Control Plasma I, High (lyophilized)
1 vial of PF4 Control Plasma II, Low (lyophilized)
3 vials of Anti-(h)-PF4-HRP Immunoconjugate (lyophilized)
1 vial of Conjugate Diluent, ready to use (25 mL)
1 vial of Wash Solution, 20 fold concentrate (50 mL)
1 vial of TMB Substrate (Peroxidase Substrate), ready to use (25 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 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). 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 at least 30 minutes before using.

1. **Microwell Strips pre-coated with anti-(h)-PF4:** 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.
2. **PF4 Sample Diluent:** Supplied ready to use, once opened, the sample diluent may be used for up to 4 weeks when stored at 2°-8°C. This sample diluent contains 0.05% Kathon CG and a rheumatoid factor inhibitor.
3. **PF4 Standard:** Reconstitute each vial with 2.0 mL of PF4 Sample Diluent. The standard concentration is approximately 10 IU/mL. See the included flyer for the exact standard concentration. Reconstituted standard is stable for at least 8 hours at room temperature (18°-25°C) or 24 hours at 2°-8°C.
4. **PF4 Control Plasma I (high):** Reconstitute this vial with 0.5 mL of filtered deionized water. This control is a high plasma control. See the enclosed data for the acceptable range. Reconstituted control is stable for 8 hours at room temperature (18°-25°C), 24 hours at 2°-8°C or 2 months at –20°C providing bacterial contamination is avoided.
5. **PF4 Control Plasma II (low):** Reconstitute this vial with 0.5 mL of filtered deionized water. This control is a low plasma control. See the enclosed data for the acceptable range. Reconstituted control is stable for 8 hours at room temperature (18°-25°C), 24 hours at 2°-8°C or 2 months at –20°C providing bacterial contamination is avoided.

6. **Anti-(h)-PF4-HRP Immunoconjugate:** Reconstitute each vial with 7.5 mL of Conjugate Diluent. Shake the vial gently to homogenize the content. Reconstituted conjugate is stable for at least 24 hours at room temperature (18°-25°C) or for at least 4 weeks 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.
8. **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 50 mL 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. Reagent contains 0.05% Kathon CG.
9. **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.
10. **Stop Solution (0.45 M Sulfuric Acid):** It is ready to use. Warning: Sulfuric acid is caustic. Handle with care. Avoid any skin and eye contact. Wear protective glasses and gloves when handling.

SPECIMEN COLLECTION AND PREPARATION

Either CTAD (citrate, theophylline, adenosine, dipyridamole) or ETP (EDTA, theophylline, prostaglandin E1) collected platelet depleted plasma may be used for this assay. Blood must be collected, with a net venipuncture without tourniquet, as follows:

1. Collect 9 parts of blood into 1 part of 3.2% (0.109M) trisodium citrate anticoagulant solution containing theophylline, adenosine and dipyridamole (CTAD) and immediately cooled.
2. Centrifuge the blood sample at 2,500 x g, at 2°-8°C, for 30 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.

Plasma must be prepared properly in order to avoid falsely elevated PF4 concentrations resulting from the presence of residual platelets or platelet activation.

Test samples must be diluted 1:2 or 1:5 in the PF4 Sample Diluent. The PF4 Sample Diluent contains a rheumatoid factor inhibitor. For expected PF4 concentrations >50 ng/mL, samples must be assayed at higher dilutions, 1:10 or 1:20. The PF4 Controls must be diluted 1:2 in the PF4 Sample Diluent.

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)

Preparation of the Standards

Using the PF4 Standard with a concentration "C" as indicated on the flyer included in the kit, prepare the following standard solutions. The PF4 Standard has been calibrated against the 1st International Standard for PF4 (NIBSC 83/505, 400 IU per ampoule).[5] One IU/mL is equivalent to 1 ng/mL.

PF4 concentration, IU/mL	C	C/2	C/5	C/10	C/20	0
Volume of PF4 Standard at "C" IU/mL	1.0 mL	0.5 mL	0.2 mL	0.1 mL	0.05 mL	0 mL
Volume of PF4 Sample Diluent	0 mL	0.5 mL	0.8 mL	0.9 mL	0.95 mL	1.0 mL

Mix gently for a complete homogenization. The standard dilutions are stable for at least 8 hours at room temperature (18°-25°C).

Assay Procedure

Remove the required number of microwell strips from the aluminium pouch sufficient for the number of assays to be performed. Place strips in the frame provided. To the appropriate wells, add reagents and perform the various assay steps as indicated on the following table:

Reagent	Volume	Procedure
PF4 standards, diluted control or diluted test sample	200 µL	Add the standard levels, diluted control or diluted 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-Human-PF4-HRP Immunoconjugate	200 µL	Add the immunoconjugate to each microwell.
Incubate for 1 hour at room temperature (18°-25°C).		

(procedure continues)

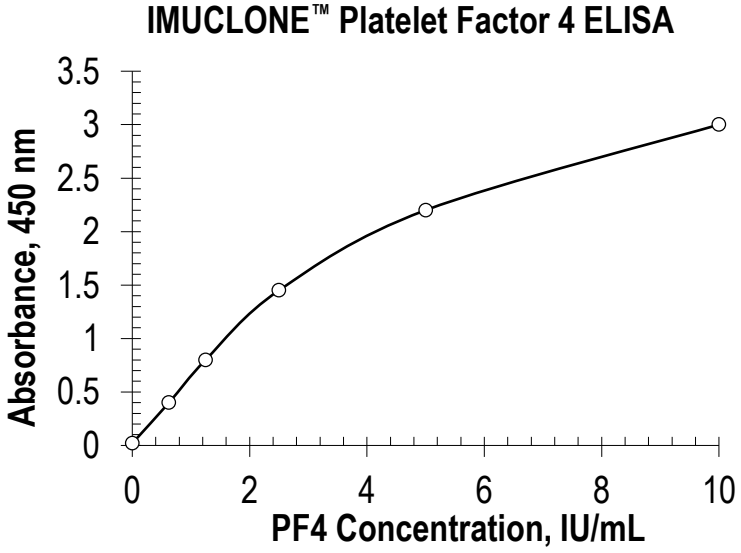
Wash Solution	300 µL	Wash the wells 5 times.
TMB Substrate	200 µL	Add the substrate immediately after washing the wells.
Incubate for exactly 5 minutes at room temperature (18°-25°C).		
Stop Solution	50 µL	Following exactly the same time intervals used for adding the substrate, stop the reaction by adding stop solution.
Wait for 10 minutes to allow the color to stabilize and measure the solution absorbance at 450 nm (A450).		

Notes:

1. Avoid leaving the plate in bright sunlight during incubations and particularly during color development.
2. Incubation temperatures of 18°-25°C must be maintained. Temperatures >25°C or <18°C will cause the measured A450 to be too high or too low, respectively.
3. Use of a microplate shaker should be limited to 1-2 minutes at the beginning of an incubation step.
4. 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.
5. 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.
6. For bichromatic absorbance readings, a reference wavelength of 690 nm or 620 nm may be used.
7. If the wash steps are not correctly performed, samples can produce a high absorbance value. In order to avoid non-specific color development, check that the wash steps are performed efficiently.

RESULTS

Construct a standard curve by plotting the mean absorbance value for each PF4 standard (ordinate) versus its corresponding concentration in IU/mL (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 generated, directly deduce the PF4 concentration in the diluted sample. To obtain the actual PF4 concentration in the sample, multiply the deduced value by the dilution factor (i.e. multiply the concentration by 2 for a 1:2 sample dilution or by 5 for a 1:5 sample dilution). Alternatively, an ELISA software (i.e. Dynex, etc.) can be used for the calculation of concentrations.

LIMITATIONS OF THE PROCEDURE

Plasma must be prepared properly in order to avoid false elevated PF4 concentrations resulting from the presence of residual platelets or platelet activation.

EXPECTED VALUES

The PF4 concentration in normal human plasma is < 10 IU/mL[6].

PERFORMANCE CHARACTERISTICS

The IMUCLONE™ PF4 ELISA measures PF4 homogeneously and is insensitive to the presence of heparin.

REFERENCES

1. Hemodson, M., et al. Isolation, crystallization, and primary amino acid sequence of human platelet factor 4. *J Biol Che*m 1977, **252 (18)**: 6276-6279.
2. Lane, D. A., et al. Anticoagulant activities of heparin oligosaccharides and their neutralization by platelet factor 4. *Biochem* 1984, **218**: 725-732.
3. Huang, SS et al. Proteoglycan carrier of human platelet factor 4.*J Biol Chem* 1982, 257: 115546-11550.
4. Zucker, M. B. and Katz, I. R. Platelet Factor 4: production, structure and physiologic and immunologic action. *Proc Soc Exp Biol Med* 1991, **98**: 693-702.
5. Kerry, P. J. and Curtis, A. D. Standardization of β-thromboglobulin (β-TG) and PF4: a collaborative study to establish international standards for β-TG and PF4. *Thromb Haemost* 1985, **53**: 51-55.
6. Kaplan, L. and Owen, J. Plasma levels of β-thromboglobulin and platelet factor 4 as indices of platelet activation in vivo. *Blood* 1981, **87**: 607-618.

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