

## IMUCLONE® Platelet Factor 4 ELISA

Product No. 634

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### 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 any biological fluid where Platelet Factor 4 may be present. The ELISA is limited to Research Use Only in the United States.

### EXPLANATION OF THE TEST

Platelet Factor 4 (PF4) is a 70 amino acid, 30,000 D molecular ratio protein. PF4 is released from activated platelet  $\alpha$  granules in a tetrameric form complexed with platelet proteoglycan<sup>1,3,4</sup>. 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 stoichiometric complex, where 1 mg of PF4 will inhibit 27 IU of heparin<sup>2</sup>.

### ASSAY PRINCIPLE

Diluted plasma samples, biological fluid or PF4 standards are added to microwells precoated with an affinity purified rabbit polyclonal antibody F(ab)<sub>2</sub> fragment 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<sub>2</sub>O<sub>2</sub>), is added to the microwell and the subsequent enzymatic reaction yields a blue colored solution. Last, the addition of sulphuric 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

6 strips of 16 antibody coated microwells (total of 96 wells) in frame holder.  
2 vials of PF4 Sample Diluent, ready to use (50 mL).  
3 vials of Human 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-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, ready to use (25 mL).  
1 vial of Stop Solution, 0.45M H<sub>2</sub>SO<sub>4</sub> (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. 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.

- F(ab)<sub>2</sub> against PF4 Coated Microwells:** 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.
- PF4 Sample Diluent:** Supplied ready to use, once opened, the diluent may be used for up to 4 weeks when stored at 2°-8°C.
- PF4 Standard:** Reconstitute each vial with 2.0 mL of PF4 Sample Diluent in order to generate a 10 IU/mL (10 ng/mL) PF4 standard. Reconstituted standard is stable for at least 8 hours at room temperature (18°-25°C) or 24 hours at 2°-8°C.
- PF4 Control I (High):** Reconstitute this vial 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.
- PF4 Control II (Low):** Reconstitute this vial 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.
- Anti-Human PF4-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 (18°-25°C) or for at least 7 days at 2°-8°C.
- Conjugate Diluent:** Supplied ready to use. Once opened, it may be used for up to 4 weeks when stored at 2°-8°C.
- 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.
- TMB Substrate:** Supplied ready to use. Once opened, it may be used for up to 4 weeks when stored at 2°-8°C.
- 0.45M H<sub>2</sub>SO<sub>4</sub>:** Supplied ready to use. **Caution:** Sulphuric acid, although diluted to 0.45M is caustic. As for any similar chemical, handle with great care. Avoid any skin and eye contact. Wear protection 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. Plasma collection should be performed as follows:

- Collect 9 parts of blood into 1 part of 3.2% (0.109M) trisodium citrate anticoagulant solution containing theophylline, adenosine and dipyridamole (CTAD).
- Centrifuge the blood sample at 2,500 rpm for 30 minutes.
- 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.
- Frozen plasma should be thawed rapidly at 37°C. Thawed plasmas should be stored at 2°-8°C and assayed within 4 hours.

Test samples must be diluted 1:2 or 1:5 in the PF4 Sample Diluent. 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.

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## PROCEDURE

Materials Provided – See Reagents

Materials Required But Not Provided

0.22 µm filtered deionized H<sub>2</sub>O

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 10 IU/mL (10 ng/mL) standard provided, prepare the following standard solutions. The PF4 standard has been calibrated against the 1<sup>st</sup> International Standard for PF4 (NIBSC 83/505, 400 IU per ampoule)<sup>5</sup>. One IU/mL is equivalent to approximately 1 ng/mL.

PF4 Concentration	10 IU/mL	5 IU/mL	2 IU/mL	1 IU/mL	0.5 IU/mL	0
Vol. of 10 IU/mL PF4 Standard	1.0 mL	0.50 mL	0.20 mL	0.10 mL	0.05 mL	0 mL
Vol. of PF4 Sample Diluent	0 mL	0.50 mL	0.80 mL	0.90 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 strips from the aluminium pouch sufficient for the number of assays to be performed. Place the strips in the frame provided. To the appropriate wells, add the reagents and perform the various assay steps as indicated on the following table:

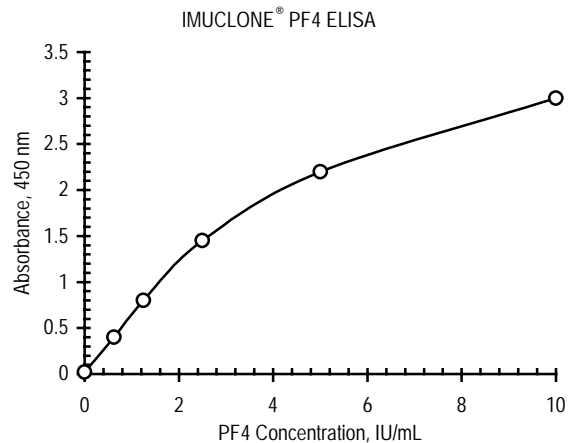
Reagent	Volume	Procedure
PF4 Standard, diluted control or diluted test sample	200 µL	Add the standard, diluted control or diluted test samples to an appropriate microwell.
Incubate for 1 hour at room temperature (18°-25°C).		
Wash Solution	300 µL	Wash the wells 5 times.
Anti-PF4-HRP Immunoconjugate	200 µL	Add the 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).		
0.45M H <sub>2</sub> SO <sub>4</sub>	50 µL	Following exactly the same time intervals used for adding the substrate, stop the reaction by adding 0.45M H <sub>2</sub> SO <sub>4</sub> .
Wait for 10 minutes to allow the color to stabilize and measure the solution absorbance at 450 nm.		

Notes:

1. Avoid letting the plate in the 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 in order to prevent the microwells from drying, which could damage the immobilized components. If necessary, fill the microwells with Wash Solution and empty it 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.

## 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

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.

### EXPECTED VALUES

The PF4 concentration in normal human plasma is < 10 IU/mL<sup>6</sup>. Elevated levels are observed in various situations associated with platelet activation such as cardiovascular disease, surgery, extra corporeal circulation, diabetes and malignant diseases<sup>6,7</sup>. Heparin administration causes PF4 to be released from its endothelial cell storage sites, greatly increasing its concentration in blood.

### PERFORMANCE CHARACTERISTICS

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

### REFERENCES

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5. Kerry, P. J. and Curtis, A. D. Standardization of β-thromboglobulin (β-TG) and platelet factor 4 (PF4): a collaborative study to establish international standards for β-TG and PF4. *Thromb Haemost* 1985, **53**: 51-55.
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7. Frischi, J., *et al.* Platelet aggregation, β-thromboglobulin and platelet factor 4 in diabetes mellitus and in patients with vasculopathy. *Thromb Haemost* 1984, **10**: 264-269.

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