

# Datasheet for ABIN1116569

# **PF4 ELISA Kit**





## Overview

Quantity:	96 tests
Target:	PF4
Reactivity:	Mouse
Method Type:	Sandwich ELISA
Detection Range:	6.25 ng/mL - 400 ng/mL
Minimum Detection Limit:	6.25 ng/mL
Application:	ELISA
Product Details	
Purpose:	The kit is a sandwich enzyme immunoassay technique for the in vitro quantitative measurement in various sample types.
Sample Type:	Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This kit recognizes natural and recombinantMousePF4. No significant cross-reactivity or interference between MousePF4 and analogues was observed. Note: Limited by existing techniques, cross reaction may still exist, as it is impossible for us to complete the cross-reactivity detection between MousePF4 and all the analogues.
Cross-Reactivity (Details):	No significant cross-reactivity or interference between mouse Platelet Factor 4 and analogues was observed. Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between Human bFGF and all the analogues, therefore, cross

#### **Product Details**

	reaction may still exist.
Sensitivity:	3.75 ng/mL
Components:	Pre-coated, ready to use 96-well strip plate, flat buttom
	Plate sealer for 96 wells
	Reference Standard
	Reference Standard & Sample Diluent
	Biotinylated Detection Antibody (100 x concentrate)
	HRP Conjugate (100 x concentrate)
	Biotinylated Detection Antibody Diluent
	HRP Conjugate Diluent
	Substrate Reagent
	Stop Solution
	Wash Buffer (25 x concentrate)
	Instruction manual

# **Target Details**

Target:	PF4
Alternative Name:	Platelet Factor 4 (PF4 Products)

# **Application Details**

#### Application Notes:

**ELISA Plate:** The just opened ELISA Plate may appear water-like substance, which is normal and will not have any impact on the experiment results.

**Add Sample:** The interval of sample adding between the first well and the last well should not be too long, otherwise will cause different pre-incubation time, which will significantly affect the experiment's accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. Parallel measure ment is recommended.

**Incubation:** To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Do not let the strips dry at any time during the assay. Strict compliance with the given incubation time and temperature.

**Washing:** The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be pat dry against absorbent paper in the washing process. But don't put absorbent paper into reaction wells directly. Note that clear the residual liquid and fingerprint in the bottom before measurement, so as not to affect the micro-titer plate reader.

Reagent Preparation: As the volume of Detection Ab and HRP Conjugate is very small, liquid may adhere to the tube wall or tube cap when being transported. You better hand-throw it or centrifugal it for 1 minute at 1000rpm. Please pipette the solution for 4-5 times before pippeting. Please carefully reconstitute Standards, working solutions of Detection Ab and HRP Conjugate according to the instructions. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10µL for once pipetting. Do not reuse standard solution, working solution of Detection Ab and HRP Conjugate, which have been diluted. If you need to use standard repeatedly, you can divide the standard into small pack according to the amount of each assay, keep them at -20°C to -80°C and avoid repeated freezing and thawing.

**Reaction Time Control:** Please control reaction time strictly following this product description! **Substrate:** Substrate Solution is easily contaminated. Please protect it from light. Stop Solution:

As it is an acid solution, please pay attention to the protection of your eyes, hands, face and clothes when using this solution.

**Mixing:** You'd better use micro-oscillator at the lowest frequency, as sufficient and gentle mixing is particularly important to reaction result. If there is no micro-oscillator available, you can knock the ELISA plate frame gently with your finger before reaction.

**Security:** Please wear lab coats and latex gloves for protection. Especially detecting samples of blood or other body fluid, please perform following the national security columns of biological laboratories.

Do not use component from different batches of kit(washing buffer and stop solution can be an exception)

To avoid cross-contamination, change pipette tips between adding of each standard level, between sample adding, and between reagent adding. Also, use separate reservoirs for each reagent. Otherwise, the results will be inaccurate!

Comment:

Information on standard material:

The formulation of the standard is 0.01 M PBS. The standard contains additives (1 % BSA).

Information on reagents:

Reagents include 1 M SO<sub>2</sub>. Azide, thimerosal, 2-mercaptoethanol (2-ME) or any other poisonous materials are not used.

Information on antibodies:

The provided antibodies and their host vary in different kits. All antibodies are affinity purified

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Sample Volume:

100 µL

Plate:

Pre-coated

Protocol:

- 1. Add 100 µL standard or sample to each well. Incubate for 90 min at 37 °C.
- 2. Remove the liquid. Add 100 µL Biotinylated Detection Antibody. Incubate for 1 hour at 37 °C.
- 3. Aspirate and wash 3 times.
- 4. Add 100 µL HRP Conjugate. Incubate for 30 min at 37 °C.
- 5. Aspirate and wash 5 times.
- 6. Add 90 µL Substrate Reagent. Incubate for 15 min at 37 °C.
- 7. Add 50 µL Stop Solution. Read at 450 nm immediately.
- 8. Calculation of results.

#### Reagent Preparation:

- 1. Bring all reagents to room temperature (18-25 °C) before use. If the kit will not be used up in one assay, please only take out the necessary strips and reagents for present experiment, and store the remaining strips and reagents at required condition.
- 2. Wash Buffer: Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40 °C water bath and mix it gently until the crystals have completely dissolved.
- 3. Standard working solution: Centrifuge the standard at 10,000xg for 1 min. Add 1.0 mL of Reference Standard &Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 400 ng/mL(or add 1.0 mL of Reference Standard &Sample Diluent, let it stand for 1-2 min and then mix it thoroughly with a vortex meter of low speed. Bubbles generated during vortex could be removed by centrifuging at a relatively low speed). Then make serial dilutions as needed. The recommended dilution gradient is as follows: 400, 200, 100, 50, 25, 12.5, 6.25, 0 ng/mL. Dilution method: Take 7 EP tubes, add 500µL of Reference Standard & Sample Diluent to each tube. Pipette 500µL of the 400 ng/mL working solution to the first tube and mix up to produce a 200 ng/mL working solution. Pipette 500µL of the solution from the former tube into the latter one according to this step. The illustration below is for reference. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube.
- 4. Biotinylated Detection Antibody working solution: Calculate the required amount before the experiment (100 μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated Biotinylated Detection Antibody at 800xg for 1 min, then dilute the 100x Concentrated Biotinylated Detection Antibody to 1x working solution with Biotinylated Detection Antibody Diluent(Concentrated Biotinylated Detection Antibody: Biotinylated Detection Antibody Diluent= 1: 99).
- 5. HRP Conjugate working solution: Calculate the required amount before the experiment (100

 $\mu$ L/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP Conjugate at 800xg for 1 min, then dilute -6- the 100x Concentrated HRP Conjugate to 1x working solution with HRP Conjugate Diluent(Concentrated HRP Conjugate: HRP Conjugate Diluent= 1: 99). 400 200 100 50 25 12.5 6.25 0

Restrictions:

For Research Use only

## Handling

Handling Advice:

All the reagents in the kit should be stored according to the labels on vials. Unused wells should be returned to the foil pouch with the desiccant pack and resealed along entire edge of zip-seal. Substrate Reagent shouldn't be kept at -20 °C (Check!). Exposure of reagents to strong light should be avoided in the process of incubation and storage. All the taps of reagents should be tightened to prevent evaporation and microbial contamination. If not to store reagents according to above suggestions, erroneous results may occur.

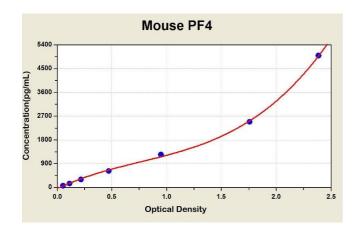
Storage:

4 °C/-20 °C

Storage Comment:

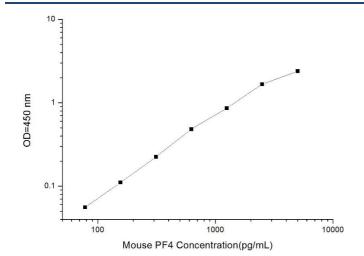
The unopened kit can be stored at 4°C for 1 month. If the kit is not used within 1 month, store the items separately according to the conditions since the kit is received.

## **Images**



### **ELISA**

**Image 1.** Diagramm of the ELISA kit to detect Mouse PF4with the optical density on the x-axis and the concentration on the y-axis.



# ELISA

Image 2. Typical standard curve