APA172Mu01 10μg Active Platelet Factor 4 (PF4) Organism Species: *Mus musculus (Mouse) Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression. Host: E. coli Residues: Val30~Ser105 Tags: N-terminal His-tag **Purity: >92%** Endotoxin Level: <1.0EU per 1mL (determined by the LAL method). Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.01% sarcosyl and 5% trehalose. Original Concentration: 150µg/mL Applications: Cell culture; Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 9.4 Predicted Molecular Mass: 12.1kDa Accurate Molecular Mass: 13kDa as determined by SDS-PAGE reducing conditions. [USAGE] Reconstitute in ddH₂O to a concentration \leq 0.1mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

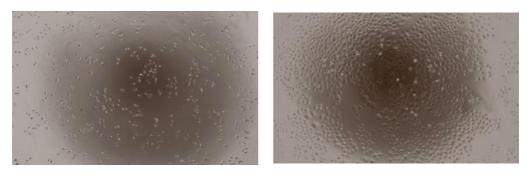
Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[<u>SEQUENCE</u>]

V TSAGPEESDG DLSCVCVKTI SSGIHLKHIT SLEVIKAGRH CAVPQLIATL KNGRKICLDR QAPLYKKVIK KILES

[ACTIVITY]

Platelet factor 4 (PF4) is a small cytokine belonging to the CXC chemokine family that is also known as chemokine (C-X-C motif) ligand 4 (CXCL4). This chemokine is released from alpha-granules of activated platelets during platelet aggregation, and promotes blood coagulation by moderating the effects of heparin-like molecules. Due to these roles, it is predicted to play a role in wound repair and inflammation. To measure its ability to inhibit the FGF basic-dependent proliferation of HUVEC human umbilical vein endothelial cells, HUVEC cells were seeded into 96-well plates at a density of 3,000 cells/well with 2% serum standard DMEM including 1µg/mL recombinant human FGF1 and various concentrations of recombinant human PF4. After incubated for 48h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10µL of CCK-8 solution was added to each well of the plate, then the absorbance at 450nm was measured using a microplate reader after incubating the plate for 1-2 hours at 37°C. Proliferation of HUVEC cells after incubation with PF4 for 48h observed by inverted microscope was shown in Figure 1. Cell viability was assessed by CCK-8 (Cell Counting Kit-8) assay after incubation with recombinant human PF4 for 48h. The result was shown in Figure 2. It was obvious that PF4 significantly FGF basic-dependent proliferation of HUVEC cells. The ED50 is 3.4µg/mL.



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Figure 1. Inhibition of HUVEC cells proliferation after stimulated with PF4.

(A) HUVEC cells cultured in DMEM with 1 μ g/mL FGF1, stimulated with 5 μ g/mL PF4 for 48h;

(B) Unstimulated HUVEC cells cultured in DMEM with 1 μ g/mL FGF1 for 48h.

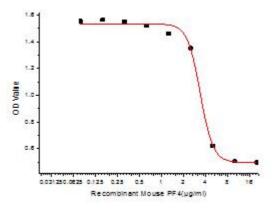
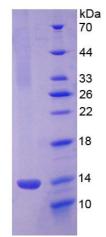
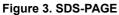


Figure 2. Inhibition of FGF basic-dependent HUVEC proliferation after stimulated with

PF4.

[IDENTIFICATION]





Sample: Active recombinant PF4, Mouse

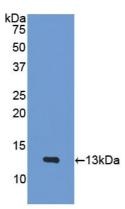


Figure 4. Western Blot

Sample: Recombinant PF4, Mouse;

Antibody: Rabbit Anti-Mouse PF4 Ab (PAA172Mu01)

[IMPORTANT NOTE]

The kit is designed for in vitro and research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.