

APB317Mu03 100µg

Active Perforin 1 (PRF1)

Organism Species: *Mus musculus* (Mouse)

Instruction manual

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Pro21~Trp554 (A144C, W373C)

Tags: N-terminal His-Tag

Subcellular Location: Cell membrane, Endosome, Lysosome, Membrane, Secreted.

Purity: >80%

Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

Traits: Freeze-dried powder

Buffer formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.01% skl, 5%Trehalose.

Original Concentration: 350µg/mL

Applications: Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 8.0

Predicted Molecular Mass: 63.6kDa

Accurate Molecular Mass: 64kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/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.

[SEQUENCE]

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PCYTATRSECKQKHKFVPGVWMAGEGMDVTTLRRSGSFPVNTQRFRLRPDRTCTLCKNSLMRDA
TQRLPVAITHWRPHSSHCQRNVAAAKVHSTEGVAREAAANINNDWRVGLDVNPRPEANMRCVS
AGSHSKVANFAAEKTYQDQYNFNSDTVECRMYSFRLVQKPPLHLDFKKALRALPRNFSSTEH
AYHRLISSYGTHFITAVDLGGRI SVLTALRTCQLTLNGLTADDEVGDCLNVEAQVSIQAQASVS
SEYKACEEKKKQHKMATSFHQTYRERHVEVLGGPLDSTHDLLFGNQATPEQFSTWTASLPSNP
GLVDYSLEPLHTLLEEQNPKREALRQAI SHYIMSRARCQNC SRPCRSGQHKSSHDSQCCECQD
SKVTNQDCCPRQRLAHLVVSNFRAEHLWGDTTATDAYLKVFFGGQEFRTGVVWNNNNPRWT
DKMDFENVLLSTGGPLRVQVWDADYGWDDLLGSCDRSPHSGFHEVTCELNHGRVKFSYHAKC
LPHLTGGTCLEYAPQGLLGDPGNGRSGAVW
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[ACTIVITY]

Perforin 1 (PRF1) is a cytolytic protein found in the granules of Cytotoxic T lymphocytes (CTLs) and NK cells. Upon degranulation, perforin inserts itself into the target cell's plasma membrane, forming a pore. The lytic membrane-inserting part of perforin is the MACPF domain. This region shares homology with cholesterol-dependent cytolysins from Gram-positive bacteria. It has been demonstrated that PRF1 causes the lysis of abnormal body cells and the elimination of virus-infected cells and tumors. To test the effect of PRF1 on cell apoptosis, Hela cells were seeded into triplicate wells of 96-well plates and allowed to attach, replaced with various concentrations of recombinant mouse PRF1. After incubated for 72h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8).

Briefly, 10 μ l of CCK-8 solution was added to each well of the plate, then the absorbance at 450 nm was measured using a microplate reader after incubating the plate for 1-4 hours at 37 $^{\circ}$ C. Cell viability was assessed by CCK-8 assay after incubation with recombinant mouse PRF1 for 72h. The result was shown in Figure 1. It was obvious that PRF1 significantly decreased cell viability of Hela cells. The ED₅₀ of recombinant mouse PRF1 is 3.205 μ g/ml.

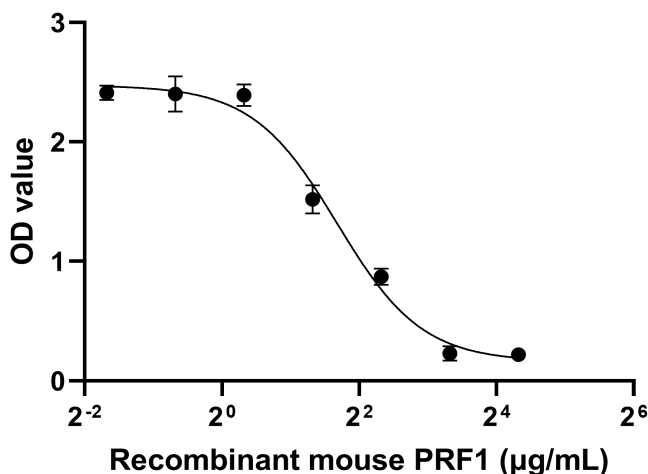


Figure 1. The dose-effect curve of recombinant mouse PRF1 on Hela cells

[IDENTIFICATION]

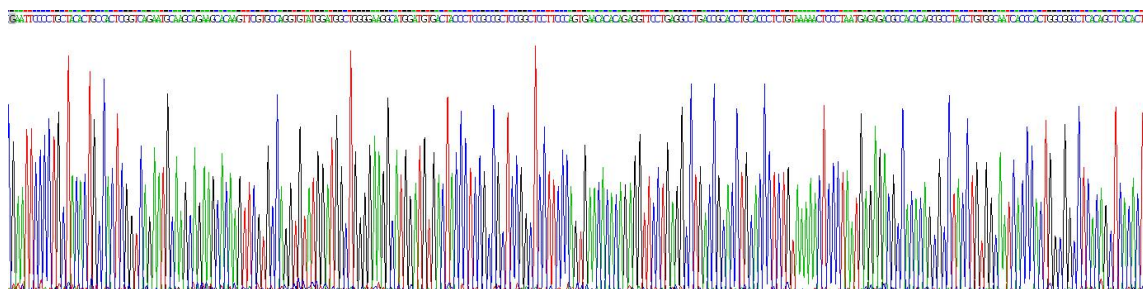


Figure 2. Gene Sequencing (extract)

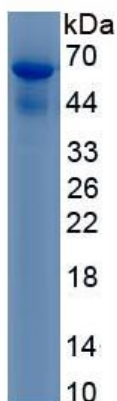


Figure 3. SDS-PAGE

Sample: Active recombinant PRF1, Mouse

[IMPORTANT NOTE]

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