



APB317Mu02 50µg

Active Perforin 1 (PRF1)

Organism Species: *Mus musculus* (Mouse)

Instruction manual

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Val40~Lys355

Tags: N-terminal His Tag

Purity: >90%

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

Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5% Trehalose.

Original Concentration: 200µg/mL

Applications: Cell culture; Activity Assays.

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

Predicted isoelectric point: 8.3

Predicted Molecular Mass: 39.0kDa

Accurate Molecular Mass: 39kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 10mM PBS (pH7.4) 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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V WMAGEGMDVT  
TLRRSGSFPV NTQRFLRPDR TCTLCKNSLM RDATQQLPVA ITHWRPHSSH  
CQRNVAAAKV HSTEGVAREA AANINNDWRV GLDVNPRPEA NMRASVAGSH  
SKVANFAAEK TYQDQYNFNS DTVECRMYSF RLVQKPLHL DFKKALRALP  
RNFNSSTEHA YHRLISSYGT HFITAVDLGG RISVLTALRT CQLTLNGLTA  
DEVGDCLNVE AQSIGAQAS VSSEYKACEE KKKQHKMATS FHQTYRERHV  
EVLGGPLDST HDLLFGNQAT PEQFSTWTAS LPSNPGLVDY SLEPLHTLLE  
EQNPK
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[ACTIVITY]

Perforin 1 (PRF1) is a pore forming cytolytic protein found in the granules of cytotoxic T lymphocytes (CTLs) and NK cells. Upon degranulation, perforin binds to the target cell's plasma membrane, and oligomerises in a Ca²⁺ dependent manner to form pores on the target cell. The pore formed allows for the passive diffusion of a family of pro-apoptotic proteases, known as the granzymes, into the target cell. The activity of recombinant PRF1 was measured by lysis of erythrocytes using a hemolysis assay. A general procedure is as follows: two-fold dilute the recombinant mouse PRF1 with 0.9% NaCl, add 50µl a serial dilution of PRF1, 10µl 0.1M CaCl₂ to each well, then add 50µl 0.25% rabbit erythrocyte (RaE) to each well and mixed gently. Add 50µl 0.9% NaCl to replace PRF1 in control wells. The plate is incubated for 20 hours at 37 °C, 5% CO₂. The results are shown in Figure 1. It was obvious that the minimal effective concentration of PRF1 is 5µg/ml.

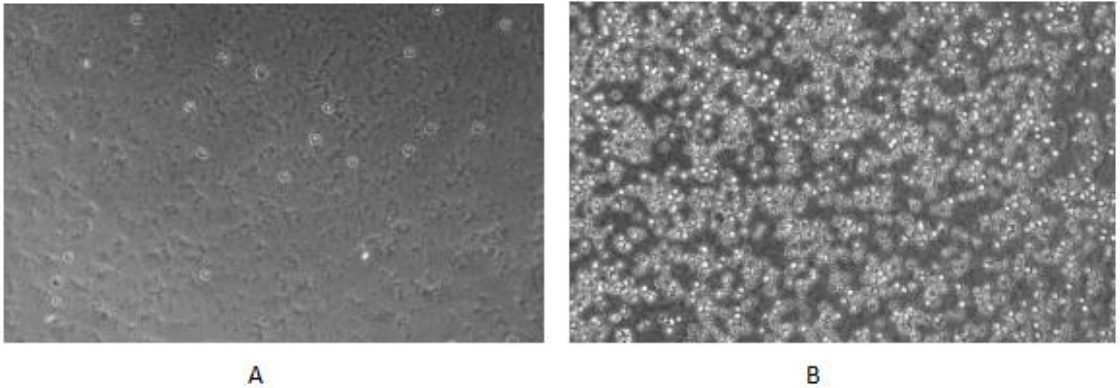


Figure 1. Hemolysis activity of recombinant mouse PRF1

[IDENTIFICATION]

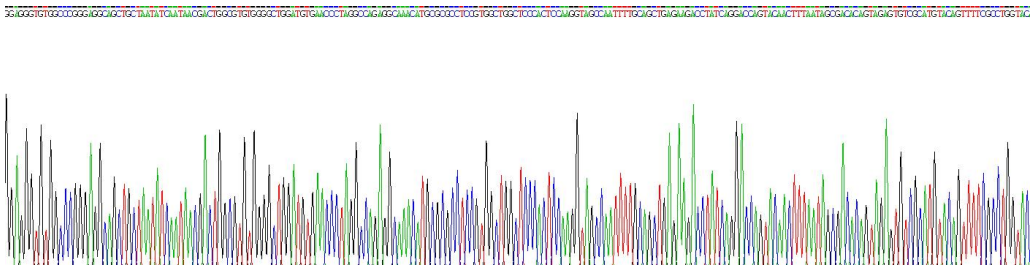


Figure 2 . Gene Sequencing (extract)

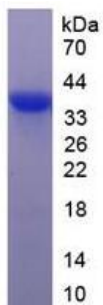


Figure 3. SDS-PAGE

Sample: Active recombinant PRF1, Mouse

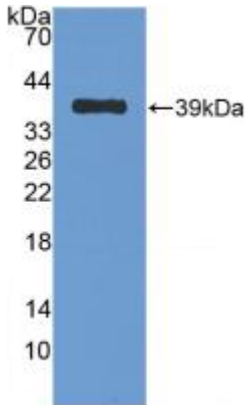


Figure 4. Western Blot

Sample: Recombinant PRF1, Mouse;

Antibody: Rabbit Anti-Mouse PRF1 Ab (PAB317Mu02)

[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.