

**APB317Mu01 50µg**  
**Active Perforin 1 (PRF1)**  
**Organism Species: *Mus musculus* (Mouse)**  
***Instruction manual***

FOR RESEARCH USE ONLY  
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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1th Edition (Apr, 2016)

## **[ PROPERTIES ]**

**Source:** Prokaryotic expression.

**Host:** *E. coli*

**Residues:** Val40~Lys355

**Tags:** Two N-terminal Tags, His-tag and GST-tag.

**Purity:** >92%

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

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

**Applications:** Cell culture; Activity Assays.

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

**Predicted isoelectric point:** 7.4

**Predicted Molecular Mass:** 65.3kDa

**Accurate Molecular Mass:** 65kDa as determined by SDS-PAGE reducing conditions.

## **[ USAGE ]**

Reconstitute in ddH<sub>2</sub>O 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 ]**

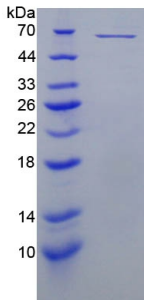
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V WMAGEGMDVT
TLRRSGSFPV NTQRFLRPDR TCTLCKNSLM RDATQRLPVA ITHWRPHSSH
CQRNVAAAKV HSTEGVAREA AANINNDWRV GLDVNPRPEA NMRASVAGSH
SKVANFAAEK TYQDQYNFNS DTVECRMYSF RLVQKPPHL DFKKALRALP
RNFNSSTEHA YHRLISSYGT HFITAVDLGG RISVLTALRT CQLTLNGLTA
DEVGDCLNVE AQVSIGAQAS VSSEYKACEE KKKQHKMATS FHQTYRERHV
EVLGGPLDST HDLLFGNQAT PEQFSTWTAS LPSNPGLVDY SLEPLHTLLE
EQNPK
```

## **[ ACTIVITY ]**

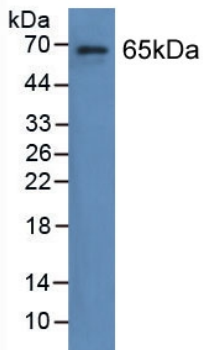
Perforin 1 (PRF1) is a pore forming cytolytic protein found in the granules of cytotoxic T lymphocytes (CTLs) and NK cells. Upon degranulation, perforin binds to the target cell's plasma membrane, and oligomerises in a Ca<sup>2+</sup> dependent manner to form pores on the target cell. The pore formed allows for the passive diffusion of a family of pro-apoptotic proteases, known as the granzymes, into the target cell. The activity of recombinant PREF1 was measured by lysis of erythrocytes using a hemolysis assay. A general procedure is as follows: two-fold dilute the recombinant mouse PREF1 with 0.9% NaCl, add 50µl a serial dilution of PREF1, 10µl 0.1M CaCl<sub>2</sub> to each well, then add 50µl 0.25% rabbit erythrocyte (RaE) to each well and mixed gently. Add 50µl 0.9% NaCl to replace PREF1 in control wells. The plate is incubated for 20 hours at 37 °C, 5% CO<sub>2</sub>. The results are shown in Figure 1. It was obvious that the minimal effective concentration of PREF1 is 2.5µg/ml.





**Figure 3. SDS-PAGE**

**Sample: Active recombinant PRF1, Mouse**



**Figure 4. Western Blot**

**Sample: Recombinant PRF1, Mouse;**

**Antibody: Rabbit Anti-Mouse PRF1 Ab (PAB317Mu01)**

### **[ 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.