

**APB317Hu02 100µg**  
**Active Perforin 1 (PRF1)**

**Organism Species: *Homo sapiens (Human)***  
***Instruction manual***

FOR RESEARCH USE ONLY  
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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1st Edition (Apr, 2016)

## **[ PROPERTIES ]**

**Source:** Prokaryotic expression.

**Host:** *E. coli*

**Residues:** Pro22~Trp555

**Tags:** N-terminal His-tag

**Purity:** >80%

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

**Buffer Formulation:** 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 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:** 7.8

**Predicted Molecular Mass:** 62.9kDa

**Accurate Molecular Mass:** 63kDa 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.

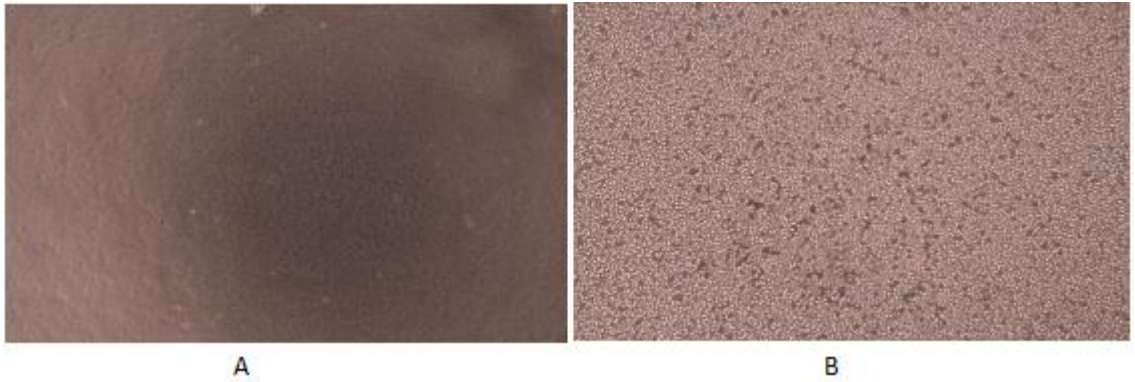
**Stability Test:** The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration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

## [ **SEQUENCE** ]

```
                PCHTAARSE CKRSHKFVPG AWLAGEGVDV
TSLRRSGSFP VDTQRFLRPD GTCTLCENAL QEGTLQRLPL ALTNWRAQGS
GCQRHVTRAK VSSTEAVARD AARSIRNDWK VGLDVTPKPT SNVHVSVAGS
HSQAANFAAQ KTHQDQYSFS TDTVECRFYS FHVVHTPPLH PDFKRALGDL
PHHFNASTQP AYLRLISNYG THFIRAVELG GRISALTALR TCELALEGLT
DNEVEDCLTV EAQVNIIGIHG SISAEAKACE EKKKKHKMTA SFHQTYRERH
SEVVGGHHTS INDLLFGIQA GPEQYSAWVN SLPGSPGLVD YTLEPLHVLL
DSQDPRREAL RRALSQYLTD RARWRDCSRP CPPGRQKSPR DPCQCVCCHGS
AVTTQDCCPR QRGLAQLEVT FIQAWGLWGD WFTATDAYVK LFFGGQELRT
STVWDMNNPI WSVRLDFGDV LLATGGPLRL QVWDQDSGRD DDLLGTCDQA
PKSGSHEVRC NLNHGHLKFR YHARCLPHLG GGTCLDYVPQ MLLGEPGNR
SGAVW
```

## [ **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 PRF1 was measured by lysis of erythrocytes using a hemolysis assay. A general procedure is as follows: two-fold dilute the recombinant human PRF1 with 0.9% NaCl, add 50µl a serial dilution of PRF1, 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 PRF1 in control wells. The plate is incubated for 20 hours at 37 °C, 5% CO<sub>2</sub>. The results are shown in Figure 2. It was obvious that the minimal effective concentration of PRF1 is 2.5µg/ml.

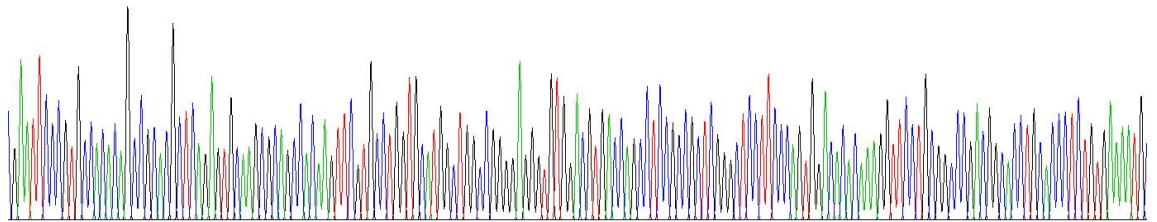


**Figure 1. Hemolysis activity of recombinant human PRF1**

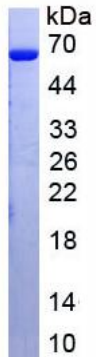
(A) 0.25% RaE treated with 2.5µg/ml PRF1 for 20h;  
 (B) 0.25% RaE treated with without PRF1.

**[ IDENTIFICATION ]**

ATGTTCCCGTGCACACAGCCCGCAGCCTCAGAGTGCAGGCGCAGCCACAGCTTCGTGCTTGTGATGGCTGGCCGCGGAGGGGTGTGGACGTGACCGCTCCGGCTCGGGCTCTCCCGCTTCCCGTGTGGACGACACAAAGGTTCTCGGGCCGAGCCCTCCACCCCTCTGTGAAAATG

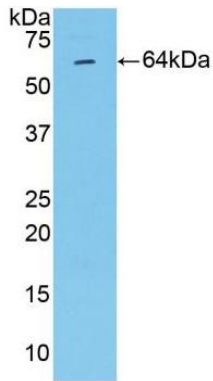


**Figure 2. Gene Sequencing (extract)**



**Figure 3. SDS-PAGE**

**Sample: Active recombinant PRF1, Human**



**Figure 4. Western Blot**

**Sample: Recombinant PRF1, Human;**

**Antibody: Rabbit Anti- Human PRF1 Ab (PAB317Hu02)**

**[ 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.