

APB317Hu02 10µg

Active Perforin 1 (PRF1)

Organism Species: *Homo sapiens (Human)*

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: 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 0.01% skl, 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: 7.8

Predicted Molecular Mass: 62.9kDa

Accurate Molecular Mass: 63kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in ddH₂O to a concentration of 0.1-0.5 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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                PCHTAARSE CKRSHKFVPG AWLAGEGVDV
TSLRRSGSFP VDTQRFLRPD GTCTLCENAL QEGTLQRLPL ALTNWRAQGS
GCQRHVTRAK VSSTEAVARD AARSIRNDWK VGLDVTPKPT SNVHVSVAGS
HSQAANFAAQ KTHQDQYSFS TDTVECRFYS FHVVHTPPLH PDFKRALGDL
PHHFNASTQP AYLRLLISNYG THFIRAVELG GRISALTALR TCELALEGLT
DNEVEDCLTV EAQVNIGIHG SISAEAKACE EKKKKHKMTA SFHQTYRERH
SEVVGHHHTS INDLLFGIQA GPEQYSAWVN SLPGSPGLVD YTLEPLHVLL
DSQDPRREAL RRALSQYLTD RARWRDCSRP CPPGRQKSPR DPCQCVCHGS
AVTTQDCCPR QRGLAQLVET FIQAWGLWGD WFTATDAYVK LFFGGQELRT
STVWDNNNPI WSVRLDFGDV LLATGGPLRL QVWDQDSGRD DDLGTCQQA
PKSGSHEVRC NLNHGHLKFR YHARCLPHLG GGTCLDYVPQ MLLGEPGGR
SGAVW
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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 human 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 2. It was obvious that the minimal effective concentration of PRF1 is 2.5µg/ml.

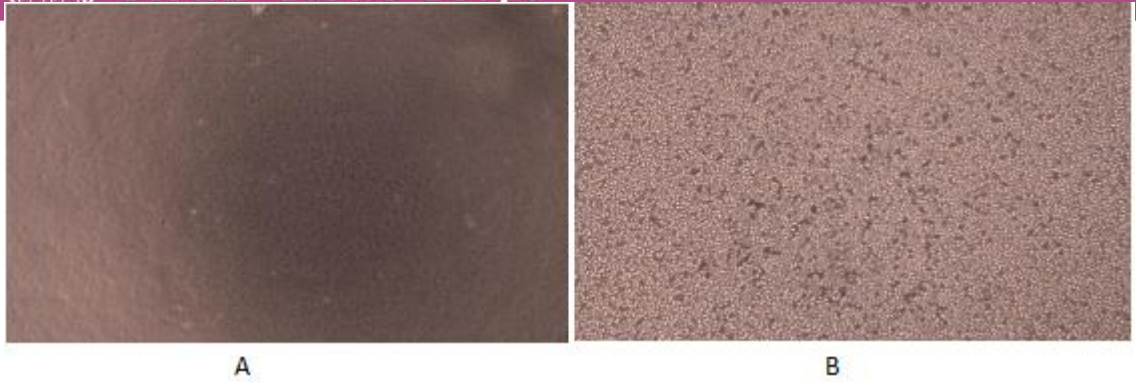


Figure 1. Hemolysis activity of recombinant human PRF1

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

[IDENTIFICATION]

ATGTTCCCGTGCACACAGCCCGCACGCTCAGAGTGCAGGCGCAGCCCAAGCTTCGTGCCTGCTGCATGGCTGGCCGCGGGAGGGGTGTGGACGTGACCGCCCTCCGGCTCCGGCTCTCCCGCTTCCCGTGTGGACACACAAAGGTTCCTCCGGCCGAGCCCTGCACCCCTCTGTGAAAATG

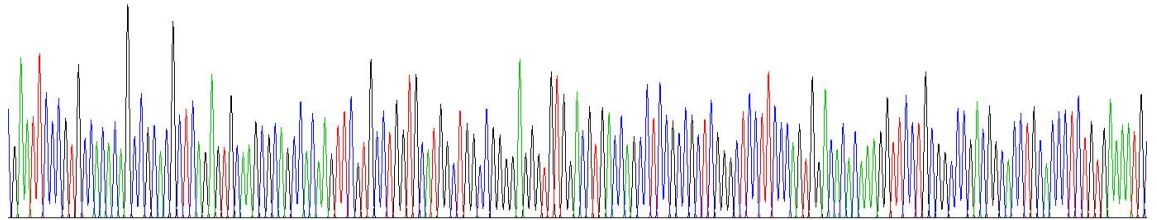


Figure 2. Gene Sequencing (extract)

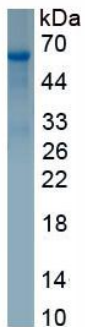


Figure 3. SDS-PAGE

Sample: Active recombinant PRF1, Human



[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.