RPU55672 200μg
Active Perforin 1 (PRF1)
Organism Species: *Homo sapiens* (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Lys32~Phe316
Tags: Two N-terminal Tags, His-tag and GST-tag
Purity: >95%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
BufferFormulation:20mMTris,150mMNaCl,pH8.0.containing0.01%skl,5%Trehalose.
Original Concentration: 250μg/mL
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 7.7
Predicted Molecular Mass: 61.5kDa
Accurate Molecular Mass: 62kDa 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 2-8ºC 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]

KRSHKFVPG AWLAGEGVDV
TSLRRSGSFP VDTQRFLRPD GTCTLCENAL QEGTLQRLPL ALTNWRAQGS
GCQRHVTRAK VSSTEAVARD AARSIRNDWK VGLDVTPKPT SNVHVSVAGS
HSQAANFAAQ KTHQDQYSFS TDTVECRFYS FHVVHTPPLH PDFKRALGDL
PHHFNASTQP AYLRLISNYG THFIRAVELG GRISALTALR TCELALEGLT
DNEVEDCLTV EAQVNIGIHG SISAEAKACE EKKKKHKMTA SFHQTYRERH
SEVVGGHHTS INDLLF

[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^{2+} 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. Besides, Calreticulin (CRT) has been identified as an interactor of PRF1, thus a binding ELISA assay was conducted to detect the interaction of recombinant human PRF1 and recombinant human CRT. Briefly, PRF1 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100μL were then transferred to CRT-coated microtiter wells and incubated for 2h at 37℃. Wells were washed with PBST and incubated for 1h with anti-PRF1 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37℃. Finally, add 50μL stop solution to the wells and read at 450nm immediately. The binding activity of PRF1 and CRT was shown in Figure 1, and this effect was in a dose dependent manner.

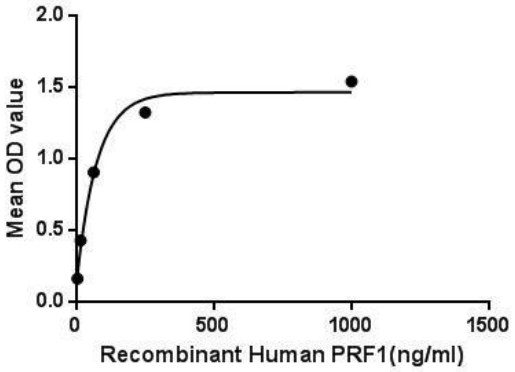

Figure 1. The binding activity of PRF1 with CRT.

The activity of recombinant PRF1 was measured by lysis of erythrocytes using a hemolysis assay. A general procedure is as fllows: two-fold dilute the recombinant human PRF1 with 0.9% NaCl, add 50μL a serial dilution of PRF1, 10μL 0.1M $CaCl_2$ to each well, then add 50μL 0.25% rabbit erythrocyte (RaE) to each well and mixed gently. Add 10μL 0.9% NaCl to reaplace $CaCl_2$ in control wells. The plate is incubated for 20 hours at 37℃, 5% CO_2. The results are shown in Figure 2. It was obvious that the minimal effective concentration of PRF1 is 12.5μg/mL.

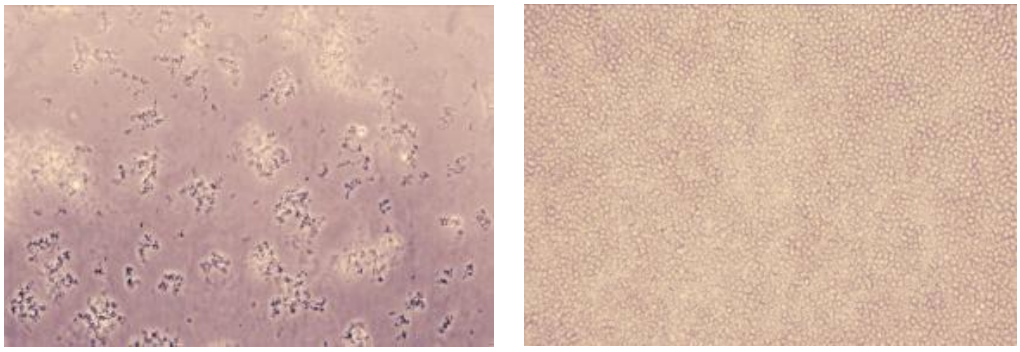

Figure 2. Hemolysis activity of recombinant human PRF1.

(A) 0.25% RaE tread with 12.5μg/mL PRF1 for 20h;

(B) Negative control (0.25% RaE tread with 12.5ug/mL PRF1) without $CaCl_2$.

[IDENTIFICATION]

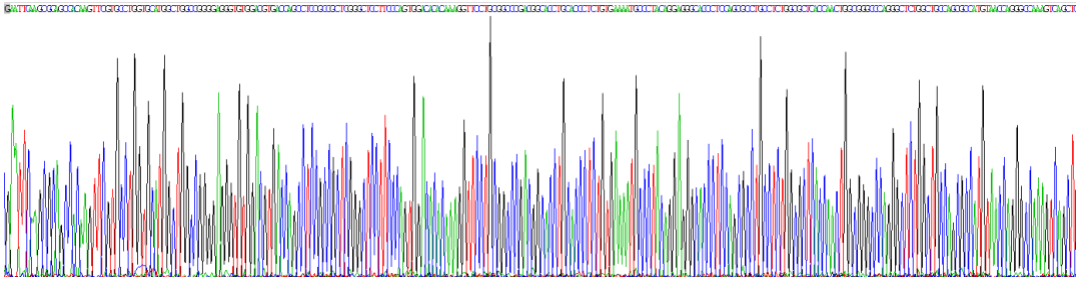

Figure 3. Gene Sequencing (extract)

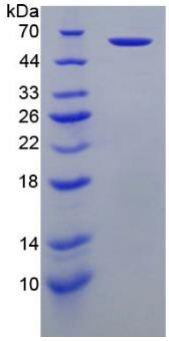

Figure 4. SDS-PAGE

Sample: Active recombinant PRF1, Human

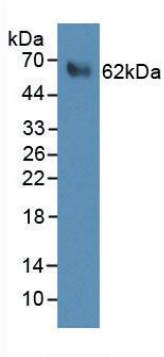

Figure 5. Western Blot

Sample: Recombinant PRF1, Human;

Antibody: Rabbit Anti-Human PRF1 Ab (PAB317Hu01)

[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.