APB317Hu01 100μg
Active Active Perforin 1 (PRF1)
Organism Species: *Homo sapiens* (Human)
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: 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).
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: 7.7
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 2-8°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 37ºC 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.

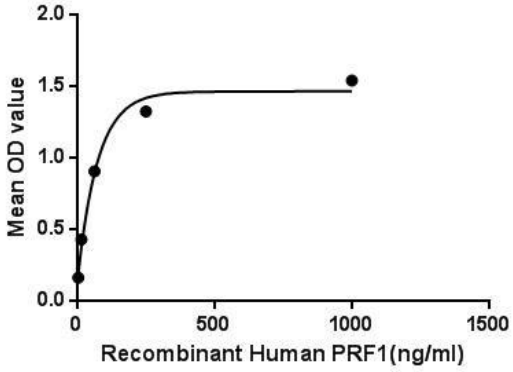

Figure 1. The binding activity of PRF1 with CRT.

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.

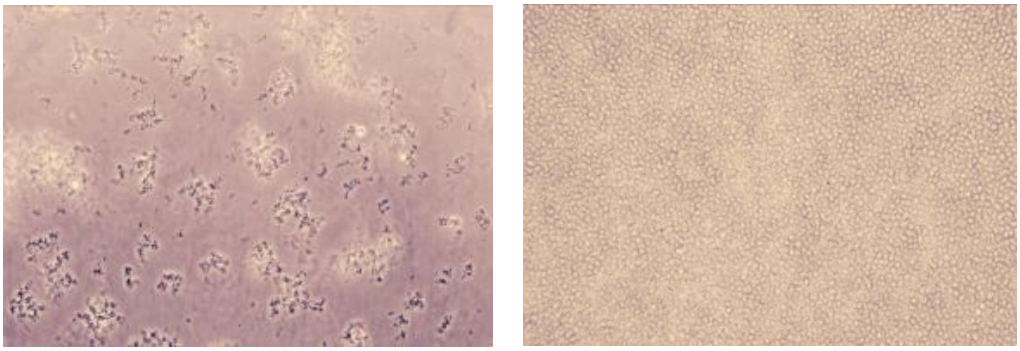

Figure 2. Hemolysis activity of recombinant human PRF1.

(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]

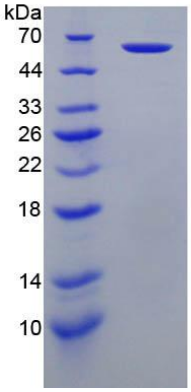

Figure 3. SDS-PAGE

Sample: Active recombinant PRF1, Human

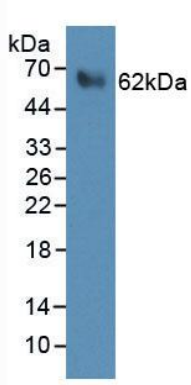

Figure 4. Western Blot

Sample: Recombinant PRF1, Human;

Antibody: Rabbit Anti-Human PRF1 Ab (PAB317Hu01)

[IMPORTANT NOTE]

The kit is designed for in vitro and research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.