Active Perforin 1 (PRF1) Instruction Manual

SBPB221Hu01

Homo sapiens (Human)

Buffer Formulation	20mM Tris, 150mM NaCl, pH8.0, containing 1mM EDTA, 1mM DTT, 0.01% SKL, 5% Trehalose and Proclin300.
Traits	Freeze-dried powder
Purity	> 95%
Isoelectric Point	7.7
Applications	Cell culture; Activity Assays.

ACTIVITY TEST

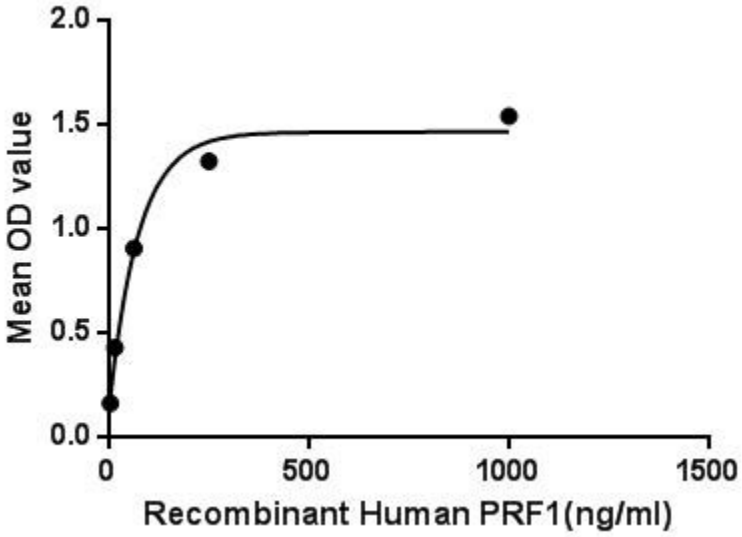

Figure. The binding activity of PRF1 with CRT.

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. 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.

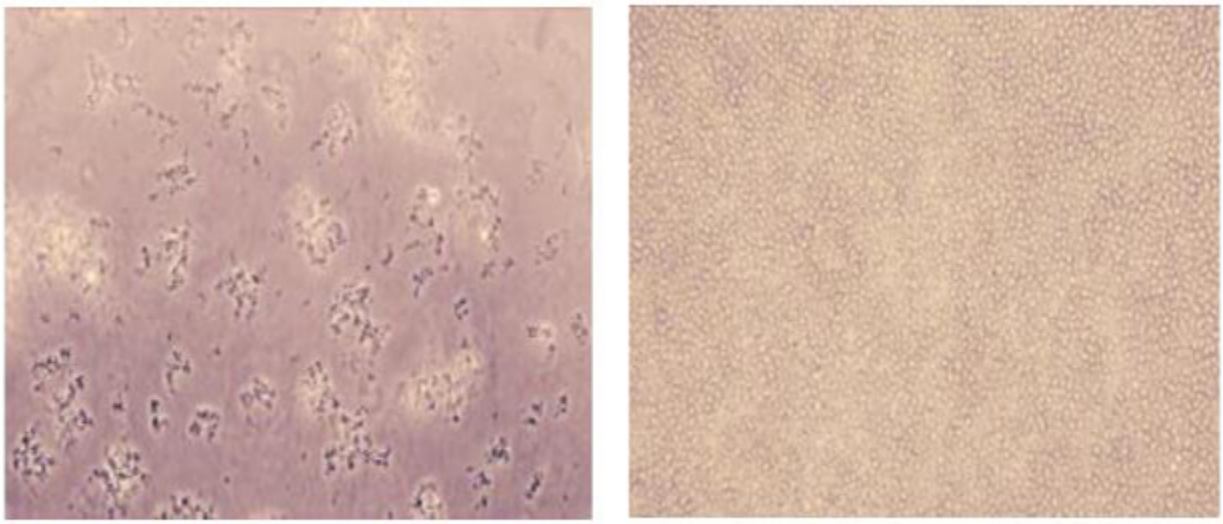

Figure 2. Hemolysis activity of recombinant human PRF1.

Figure. Hemolysis activity of recombinant human PRF1.
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 CaCl2 to each well, then add 50μL 0.25% rabbit erythrocyte (RaE) to each well and mixed gently. Add 10μL 0.9% NaCl to reaplace CaCl2 in control wells. The plate is incubated for 20 hours at 37℃, 5% CO2. The results are shown in Figure 2. It was obvious that the minimal effective concentration of PRF1 is 12.5μg/mL.
(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 CaCl2.

USAGE

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

STORAGE

Avoid repeated freeze/thaw cycles. Store at 2-8°C for one month. Aliquot and store at -80°C for 12 months.

STABILITY

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.

Image

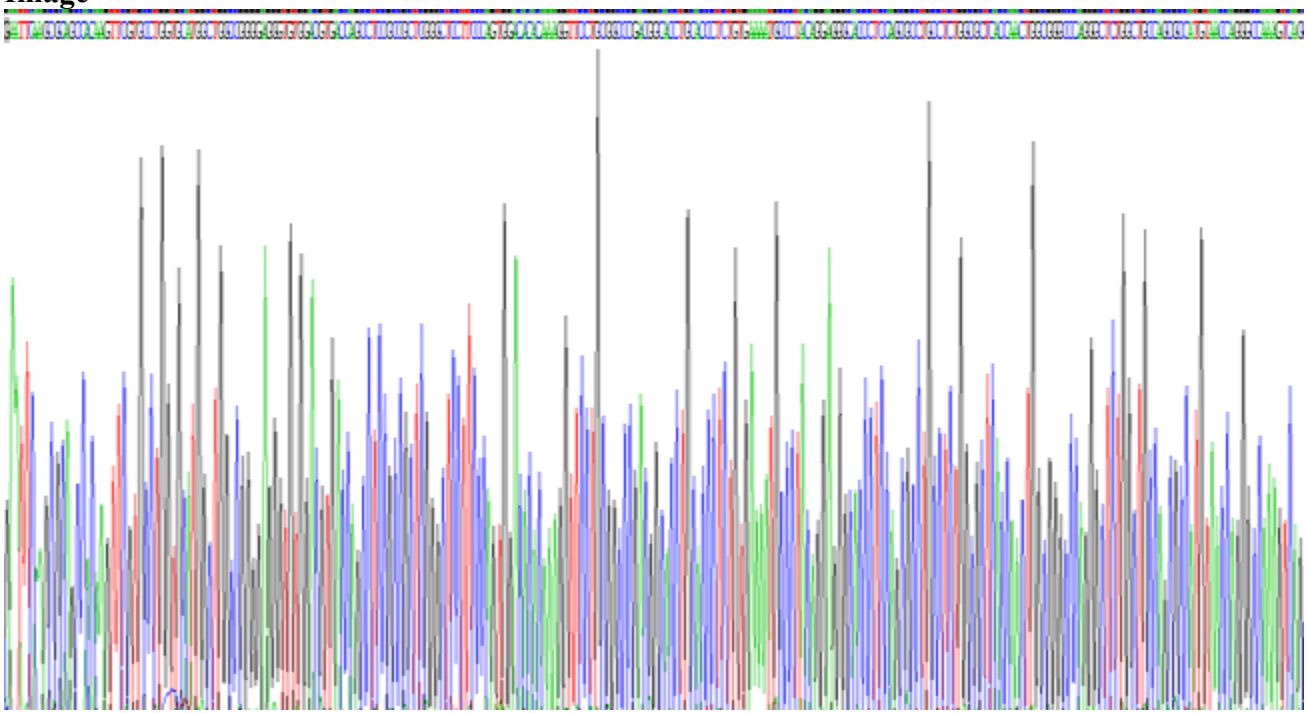

Figure. Western Blot

[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.