

Active Perforin 1 (PRF1) Instruction Manual

SBPB221Mu01

Mus musculus (Mouse)

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

> 90%

Isoelectric Point

7.4

Applications

Cell culture; Activity Assays.

ACTIVITY TEST

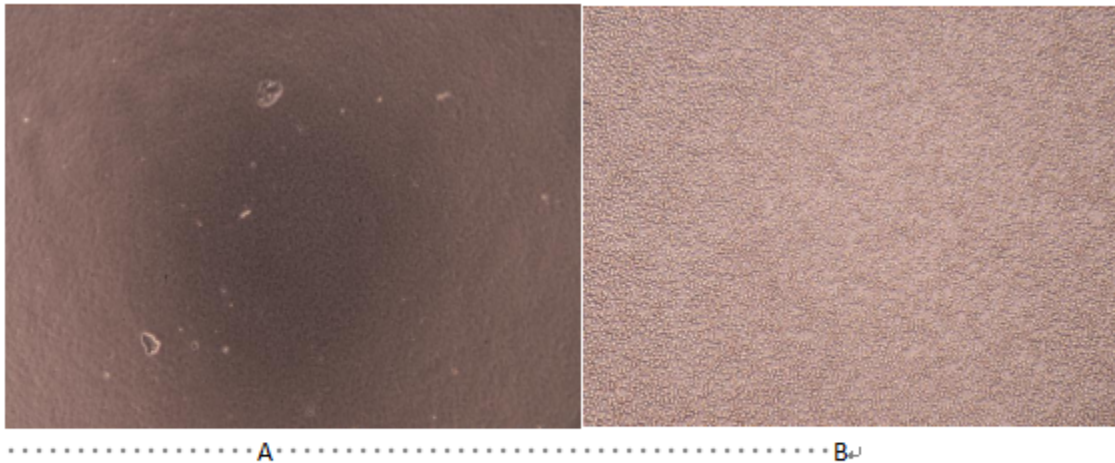


Figure 1. Hemolysis activity of recombinant mouse PRF1.

(A) 0.25% RaE treated with 25µg/ml PRF1 for 20h.

(B) 0.25% RaE treated without PRF1.

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 mouse 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

PREF1 in control wells. The plate is incubated for 20 hours at 37 °C, 5% CO₂. The results are shown in Figure 1. It was obvious that the minimal effective concentration of PREF1 is 2.5µg/ml.

V WMAGEGMDVT
TLRRSGSFPV NTQRFLRPDR TCTLCKNSLM RDATQRLPVA ITHWRPHSSH
CQRNVAAAKV HSTEGVAREA AANINNDWRV GLDVNPRPEA NMRASVAGSH
SKVANFAAEK TYQDQYNFNS DTVECRMYSF RLVQKPLHL DFKKALRALP
RNFNSSTEHA YHRLISSYGT HFITAVDLGG RISVLTALRT CQLTLNGLTA
DEVGDCLNVE AQVSIQAQAS VSSEYKACEE KKKQHKMATS FHQTYRERHV
EVLGGPLDST HDLLFGNQAT PEQFSTWTAS LPSNPGLVDY SLEPLHTLLE
EQNPK

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

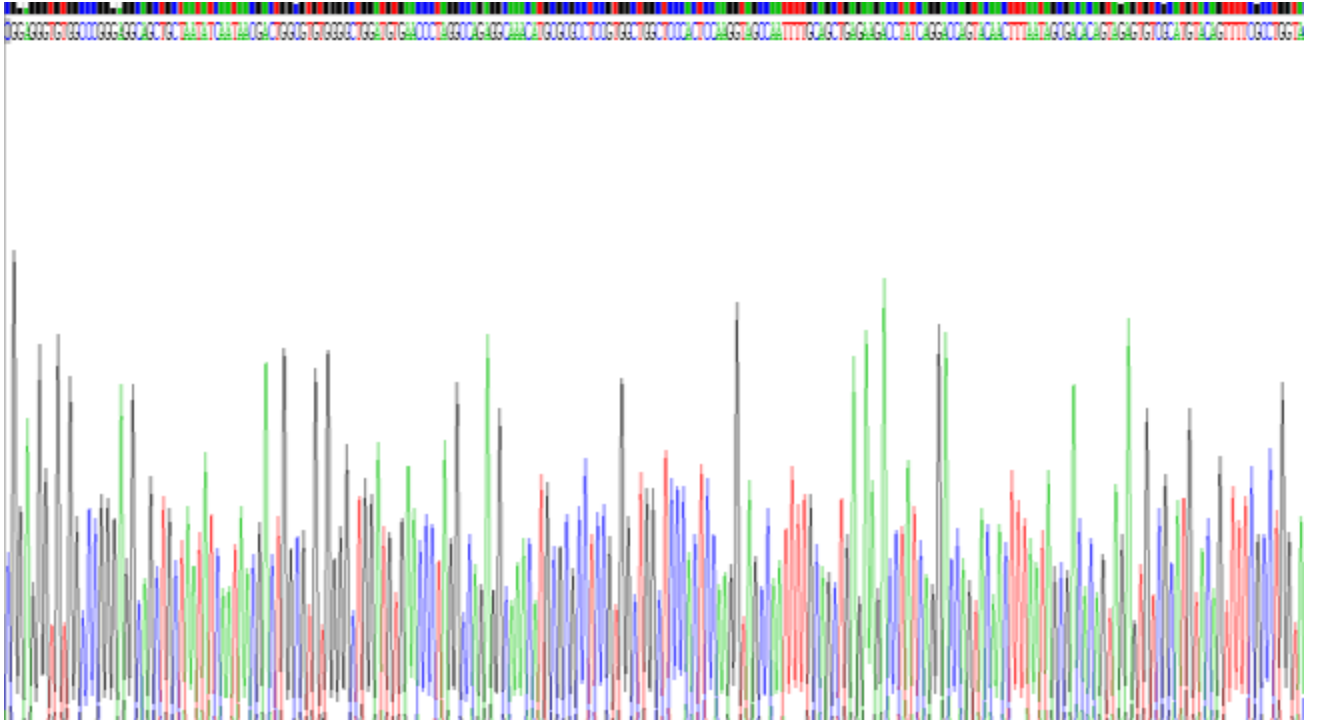


Figure . Gene Sequencing (extract)

Image

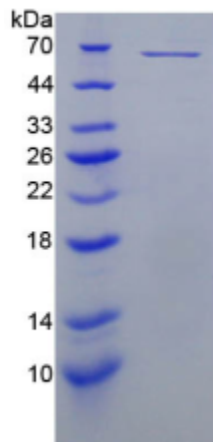


Figure. SDS-PAGE

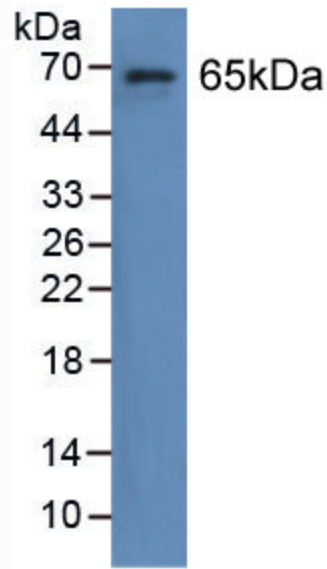


Figure. Western Blot

[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.