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RPU55673
Active Perforin 1 (PRF1)
Organism Species: *Mus musculus* (Mouse)
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: Val40~Lys355

Tags: Two N-terminal Tags, His-tag and GST-tag.

Purity: >92%

Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).

BufferFormulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.01% skl, 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: 7.4

Predicted Molecular Mass: 65.3kDa

Accurate Molecular Mass: 65kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in ddH_2O 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^{o}C for one month.
Aliquot and store at -80^{o}C for 12 months.

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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]

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

[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. Perforin is thought to act by creating holes in the plasma membrane which triggers an influx of calcium and initiates membrane repair mechanisms. These repair mechanisms bring perforin and granzymes into early endosomes. Besides, Granzyme A (GZMA) has been identified as an interactor of PRF1, thus a binding ELISA assay was conducted to detect the interaction of recombinant mouse PRF1 and recombinant mouse GZMA. Briefly, PRF1 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100µL were then transferred to GZMA-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.

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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 GZMA was shown in Figure 1, and this effect was in a dose dependent manner.

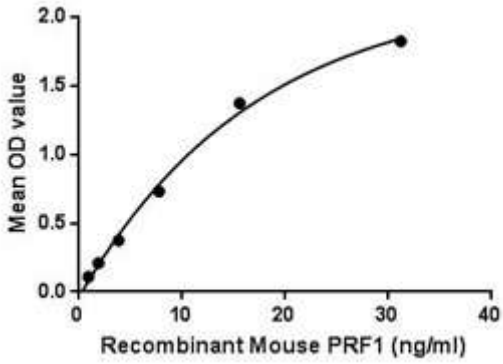

Figure 1. The binding activity of PRF1 with GZMA

[IDENTIFICATION]

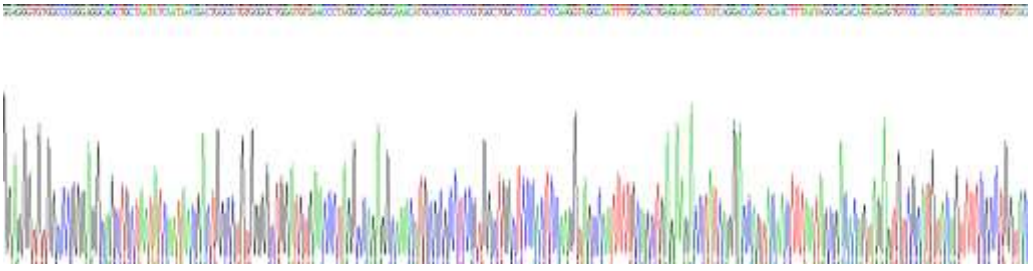

Figure 2 . Gene Sequencing (extract)

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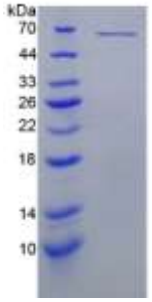

Figure 3. SDS-PAGE

Sample: Active recombinant PRF1, Mouse

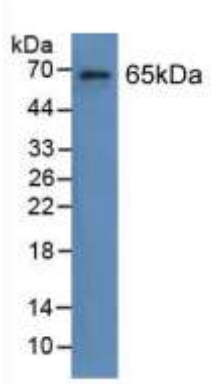

Figure 4. Western Blot

Sample: Recombinant PRF1, Mouse;

Antibody: Rabbit Anti-Mouse PRF1 Ab (PAB317Mu01)

[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.