APB317Ra01 100μg
Active Perforin 1 (PRF1)
Organism Species: ***Rattus norvegicus (Rat)***
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: Ala120~Asp353
Tags: N-terminal His-tag
Purity: >95%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, 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: 6.3
Predicted Molecular Mass: 29.5kDa
Accurate Molecular Mass: 33kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 10mM PBS (pH7.4) 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]

A
AANINNDWRA GLDVNPKPEA NVHVSVAGSH SKIANFAAEK AHQDQYNFNT DTVECRMYSF
RLAQKPPLHP DFRKALKNLP HNFNSSTEHA YRRLISSYGT HFITAVDLGG RVSVLTALRT
CQLTLDGLTA DEVGDCLSVE AQVSIGAQAS VSSEYKACEE KKKQHKIATS FHQTYRERHV
EVLGGPLDSS NDLLFGNQAT PEHFSTWIAS LPTRPDVVDY SLEPLHILLE DSD

[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. The activity of recombinant rat PRF1 was measured by lysis of erythrocytes using a hemolysis assay. A general procedure is as fllows: two-fold dilute the recombinant rat 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 50μl 0.9% NaCl to reaplace PRF1 in control wells. The plate is incubated for 20 hours at 37 ℃, 5% CO_2. The results are shown in Figure 1. It was obvious that the minimal effective concentration of PRF1 is 7.5μg/ml.

A B

Figure 1. Hemolysis activity of recombinant rat PRF1

(A) 0.25% RaE tread with 7.5μg/ml PRF1 for 20h;

(B) 0.25% RaE tread without PRF1.

[IDENTIFICATION]

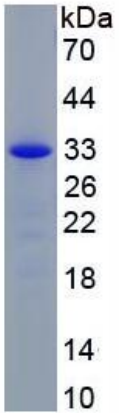

Figure 2. SDS-PAGE

Sample: Active recombinant PRF1, rat

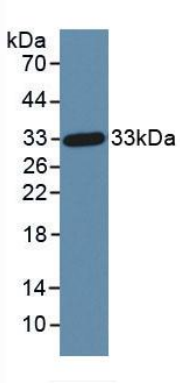

Figure 3. Western Blot

Sample: Recombinant PRF1, rat;

Antibody: Rabbit Anti- rat PRF1 Ab (PAB317Ra01)

[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.