#### APA599Hu51 50μg

**Active Granzyme A (GZMA)** 

**Organism Species: Homo sapiens (Human)** 

Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

### [PROPERTIES]

Source: Eukaryotic expression

Host: Yeast

Residues: Ile29~Val262 Tags: N-terminal His-tag

**Purity: >98%** 

**Endotoxin Level:** <1.0EU per 1μg (determined by the LAL method). **Buffer Formulation:** PBS, pH7.4, containing 0.01% SKL, 5% Trehalose.

Original Concentration: 50µg/mL

Applications: Cell culture; Activity Assays; In vivo assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 9.2

Predicted Molecular Mass: 28.2kDa

**Accurate Molecular Mass:** 28kDa as determined by SDS-PAGE reducing conditions.

### [USAGE]

Reconstitute in ddH<sub>2</sub>O to a concentration of 0-0.5 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 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]

II GGNEVTPHSR PYMVLLSLDR
KTICAGALIA KDWVLTAAHC NLNKRSQVIL GAHSITREEP TKQIMLVKKE
FPYPCYDPAT REGDLKLLQL MEKAKINKYV TILHLPKKGD DVKPGTMCQV
AGWGRTHNSA SWSDTLREVN ITIIDRKVCN DRNHYNFNPV IGMNMVCAGS
LRGGRDSCNG DSGSPLLCEG VFRGVTSFGL ENKCGDPRGP GVYILLSKKH
LNWIIMTIKG AV

### [ACTIVITY]

Granzyme A is a member of the granzyme family of the serine proteases found specifically in the cytotoxic granules of cytotoxic T lymphocytes (CTL) and natural killer (NK) cells. Granzyme A is the most abundant protease in CTL and NK cells. It induces caspase-independent cell death when introduced into target cells by perforin (1). Human granzyme A is synthesized as a precursor (262 residues) with a signal peptide (residues 1-26), a propeptide (residues 27-28) and a mature chain (residues 29-262 ) (2). The purified recombinant human Granzyme A consists of residues 29 to 262 which activity was measured by its ability to cleaves a thioester substrate Z-Lys-SBzI+HCI. The reaction was performed in 0.05 M Tris, 0.15 M NaCl, 0.01% Triton X-100, pH 8.0 (Assay Buffer), initiated by addition 50  $\mu$ L of various concentrations of GZMA (dilute by Assay Buffer) to 50  $\mu$ L of 1.2 mM Substrate and DTNB mixture. The final well serves as a negative control with no GZMA, replace with 50 $\mu$ L assay buffer. Incubated at 25 °C for 5min, then read at a wavelength of 405 nm. The specific activity of recombinant human Granzyme A is 912 pmol/min/ $\mu$ g.

#### Specific Activity (pmol/min/ug)=

## Adjusted V<sub>max</sub>\* (OD/min) x well volume (L) x 10<sup>12</sup> pmol/mol

ext. coeff\*\* (M-1cm-1) x path corr.\*\*\* (cm) x amount of enzyme (ug)

## [IDENTIFICATION]

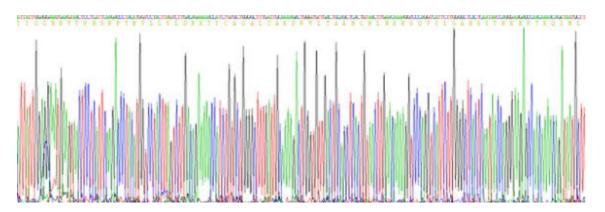


Figure 1. Gene Sequencing (extract)

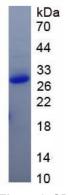


Figure 2. SDS-PAGE

Sample: Active recombinant GZMA, Human

<sup>\*</sup>Adjusted for Substrate Blank

<sup>\*\*</sup>Using the extinction coefficient 13800 M-1cm-1

<sup>\*\*\*</sup> Using the path correction 0.320 cm

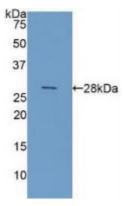


Figure 3. Western Blot

Sample: Recombinant GZMA, Human;

Antibody: Rabbit Anti-Human GZMA Ab (PAA599Hu05)

# [ 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.