

APA600Ra61 100µg

Active Granzyme B (GZMB)

Organism Species: *Rattus norvegicus* (Rat)

Instruction manual

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Eukaryotic expression.

Host: 293F cell

Residues: Gly19~Ser248

Tags: N-terminal His-tag

Purity: >80%

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

Buffer Formulation: PBS, pH7.4, containing 5% Trehalose .

Original Concentration: 200µg/mL

Applications: Activity Assays.

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

Predicted isoelectric point: 10.1

Predicted Molecular Mass: 27.0kDa

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 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]

GEIIGGHEAKPHSRPYMAYLQIMDEYSGSKKCGGLIREDFVLTAAHCSGSKINVTLGAHNIKEQE
KMQQIIPVVKIIPHPAYNSKTISNDIMLLKLKSKAKRSSAVKPLNLPARNVKVKPGDVCYVAGWGK
LGPMGKYSDTLQEVELTVQEDQKCESYLKNYFDKANEICAGDPKIKRASFRGDSGGPLVCKKVA
AGIVSYGQNDGSTPRAFTKVSTFLSWIKKTMKKS

[ACTIVITY]

Granzyme B (GZMB) is a serine protease predominantly secreted by cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells. It serves as a central effector molecule in the granule exocytosis pathway, which is essential for cell-mediated elimination of virus-infected and malignant cells. Upon target cell recognition, CTLs and NK cells release GZMB together with perforin. Perforin facilitates the entry of GZMB into the target cell by forming pores in the membrane, where GZMB then initiates apoptotic signaling. In addition to its well-established role in apoptosis, GZMB also contributes to immune regulation and tissue remodeling through involvement in inflammatory responses and extracellular matrix degradation. A key mechanism of its pro-apoptotic function involves the direct cleavage of Bid, generating truncated Bid (tBid), which potently activates the mitochondrial apoptosis pathway. To detect the activity of recombinant GZMB, a functional ELISA assay was performed to evaluate the interaction between recombinant rat GZMB and recombinant human Bid. Briefly, biotin-linked GZMB were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100µl were then transferred to Bid-coated microtiter wells and incubated for 1h at 37 °C. Wells were washed with PBST 3 times and incubation with Streptavidin-HRP for 30min, then wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 °C. Finally, add 50µl stop solution to the wells and read at 450nm immediately. The binding activity of GZMB and Bid was shown in Figure 1, the EC₅₀ for this effect is 0.16µg/mL.

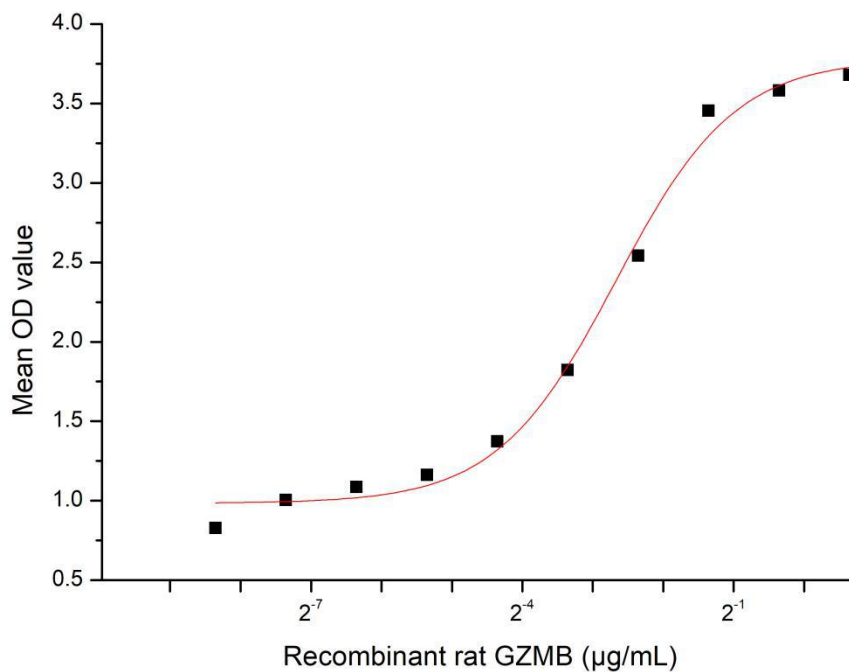


Figure 1. The binding activity of recombinant rat GZMB and recombinant human Bid

[IDENTIFICATION]

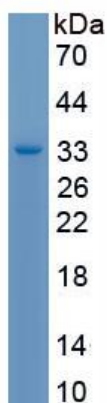


Figure 2. SDS-PAGE

Sample: Active recombinant GZMB, Rat

[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.