APB517Hu61 50μg
Active Granulysin (GNLY)
Organism Species: *Homo sapiens* (Human)
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: Arg23~Leu145
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: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 9.4
Predicted Molecular Mass: 15.6kDa
Accurate Molecular Mass: 18&16kDa as determined by SDS-PAGE reducing conditions.
Phenomenon explanation:
The possible reasons that the actual band size differs from the predicted are as follows:
1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

[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]

RLSPEYYD LARAHLRDEE KSCPCLAQEG
PQGDLLTKTQ ELGRDYRTCL TIVQKLKKMV DKPTQRSVSN AATRVCRTGR
SRWRDVCRNF MRRYQSRVTQ GLVAGETAQQ ICEDLRLCIP STGPL

[ACTIVITY]

Granulysin (GNLY) is a member of the saposin-like protein (SAPLIP) family of membrane disrupting proteins. Granulysin is expressed in granules of natural killer and activated cytotoxic T cells. It exhibits cytolytic activity against intracellular or extracellular microbes and also tumors, either alone or in synergy with perforin. Human granulysin has structural similarity and 30 - 40% aa identity to granulysins and NK-lysins of other mammals such as bovine, porcine and canine; similar peptides in rodents have not been identified. The 15 kDa unglycosylated protein contains five helical domains; helix 2 and 3 contain 9 arginines and one cysteine critical for activity. The activity of GNLY is usually measured by a cell apoptosis assay using Jurkat cells. Jurkat cells were seeded into triplicate wells of 96-well plates at a density of 8,000 cells/well with 5% serum standard 1640 which contains various concentrations of recombinant human GM-CSF. After incubated for 3

days, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10 μl of CCK-8 solution was added to each well of the plate, then the absorbance at 450 nm was measured using a microplate reader after incubating the plate for 2-4 hours at 37 ℃. Apoptosis of Jurkat cells after incubation with GNLY for 3 days observed by inverted microscope was shown in Figure 1. Cell viability was assessed by CCK-8 (Cell Counting Kit-8) assay after incubation with recombinant human GNLY for 3 days. The result was shown in Figure 2. It was obvious that GNLY significantly decreased cell viability of Jurkat cells. The ED50 is 0.84 ug/ml.

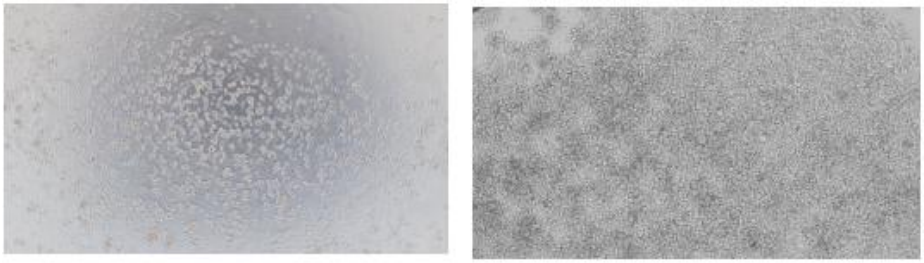

Figure 1. Inhibition of Jurkat cells proliferation after stimulated with GNLY

(A) Jurkat cells cultured in 1640, stimulated with 1.25 μg/mL GNLY for 72h;
(B) Unstimulated Jurkat cells cultured in 1640 for 72h.

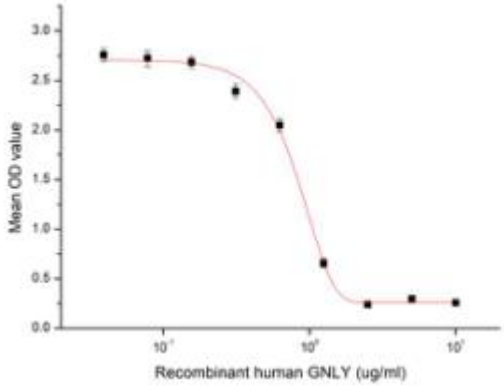

Figure 2. Inhibition of Jurkat cells proliferation after stimulated with GNLY.

[IDENTIFICATION]

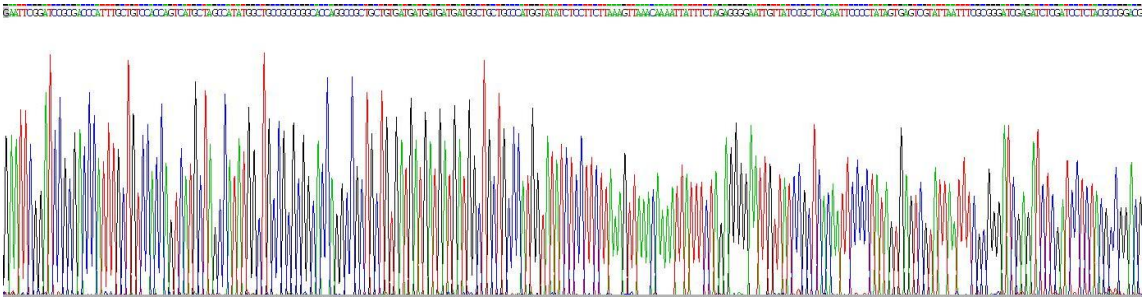

Figure 3. Gene Sequencing (extract)

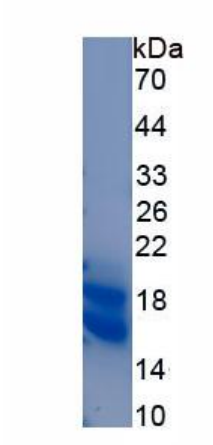

Figure 4. SDS-PAGE

Sample: Active recombinant GNLY, Human

[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.