

APA626Ra03 100µg

Active Caspase 3 (CASP3)

Organism Species: *Rattus norvegicus* (Rat)

Instruction manual

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

12th Edition (Revised in Aug, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Ser29~His277

Tags: N-terminal His-tag

Purity: >80%

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

Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 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: 6.6

Predicted Molecular Mass: 31.7kDa

Accurate Molecular Mass: 32kDa 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.

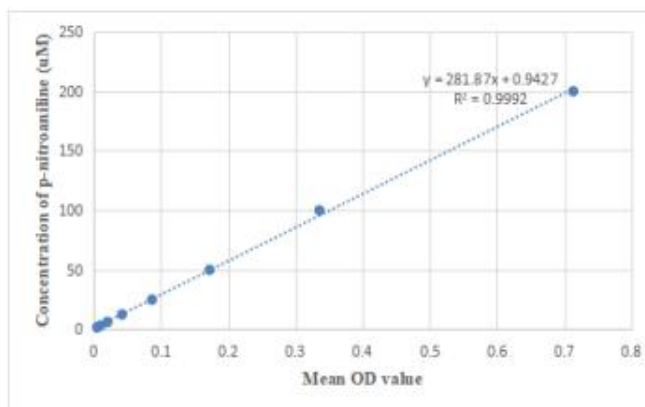
Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[**SEQUENCE**]

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SGIYLDSSYKMDYPEMGLCIIINNKNFHKSTGMSARNGTDVDAANLRETFMALKYEVNRKNDL TREEIMELMDSVSKEDHSKRSSFVFCVI  
LSHGDEGVIFGTNGPVDLKKLTSFPRGDYCRSLTGKPKLFI IQACRGTELDG IETDSGTDDDMACQKIPVEADFLYAYSTAPGYYSWRN  
SRDGSWFIQSLCAMLKLYAHKLEFMHILTRVNRKVATEFESFSLDATFHAKKQIPCIVSMLTKELYFYH
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[**ACTIVITY**]

Caspase 3 is a member of the cysteine-aspartic acid protease (caspase) family. Sequential activation of caspases plays a central role in the execution-phase of cell apoptosis. Caspases exist as inactive proenzymes that undergo proteolytic processing at conserved aspartic residues to produce two subunits, large and small, that dimerize to form the active enzyme. This protein cleaves and activates caspase 6 and 7; and the protein itself is processed and activated by caspases 8, 9, and 10. Caspase 3 can hydrolyze the peptide substrate acetyl-Asp-Glu-Val-Asp-p-nitroanilide (Ac-DEVD-pNA) resulting in the release of the p-nitroaniline (pNA) moiety. p-Nitroaniline has a high absorbance at 405 nm. Thus the activity of recombinant rat caspase 3 can be measured by calculating the concentration of the pNA released from the substrate. The reaction was performed in adding 50 μ l 2 \times buffer (50mM HEPES, 100mM NaCl, 10mM DTT, 2mM EDTA, 10% glycerol) to 96 well plates, then add 50 μ l various concentration of caspase 3 (diluted by 1 \times buffer, 25mM HEPES, 50mM NaCl, 5mM DTT, 1mM EDTA, 5% glycerol) to each well, finally, add 5 μ l 4mM Ac-DEVD-pNA to each well. Cover the 96 well plates and incubate at 37 $^{\circ}$ C for 1h. p-Nitroaniline (pNA) Standard curve prepare by double dilute 200 μ M pNA with 1 \times buffer and record the OD value at 405 nm. The specific activity of recombinant rat caspase3 is >3300 pmol/min/ μ g.



OD405nm	p-nitroaniline (uM)
0.7135	200
0.3359	100
0.1726	50
0.0869	25
0.0422	12.5
0.0207	6.25
0.01	3.125
0.005	1.5625

Figure 1. The standard curve of p-nitroaniline

One unit of enzyme activity is defined as the 1 μg of enzyme required to convert 1 pmol of Ac-DEVD-pNA to p-nitroaniline in 1min at 37°C.

$$\text{Specific Activity (pmol/min/}\mu\text{g)} = \frac{\Delta OD * F}{T * N}$$

Δ OD=Adjusted for Substrate Blank

F=Conversion Factor(convert from standard curve of p-nitroaniline)

T= Time

N=Amount of enzyme

[IDENTIFICATION]

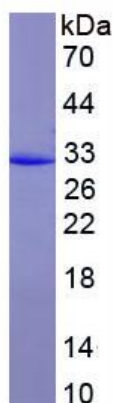


Figure 2. SDS-PAGE

Sample: Active recombinant CASP3, 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.