APA626Hu01 100μg
Active Caspase 3 (CASP3)
Organism Species: *Homo sapiens (Human)*
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: Ser29~His277
Tags: N-terminal His-tag
Purity: >90%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 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: 7.1
Predicted Molecular Mass: 32.2kDa
Accurate Molecular Mass: 14/18/20kDa 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 20mM Tris, 150mM NaCl (pH8.0) 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]

SG ISLDNSYKMD YPEMGLCIII
NNKNFHKSTG MTSRSGTDVD AANLRETFRN LKYEVRNKND LTREEIVELM
RDVSKEDHSK RSSFVCVLLS HGEEGIIFGT NGPVDLKKIT NFFRGDRCRS
LTGKPKLFII QACRGTELDC GIETDSGVDD DMACHKIPVE ADFLYAYSTA
PGYYSWRNSK DGSWFIQSLC AMLKQYADKL EFMHILTRVN RKVATEFESF
SFDATFHAKK QIPCIVSMLT KELYFYH

[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 caspases 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 405nm. Thus the activity of recombinant human caspase 3 can be measured by calculate the concentration of the pNA released from the substrate. The reaction was performed in adding 50μL 2×buffer (50mM HEPES, 100mM NaCl, 10mM DTT, 2mM EDTA, 10% glycerol) to 96 well plates, then add 50μL various concentration of caspe 3 (diluted by 1×buffer, 25mM HEPES, 50mM NaCl, 5mM DTT, 1mM EDTA, 5% glycerol) to each well, finally, add 5μL 4mmol Ac-DEVD-pNA to each well. Cover the 96 well plates and incubate at 37℃ for 2h. p-Nitroaniline (pNA) standard curve prepare by double dilute 200μM pNA with 1×buffer and record the OD value at 405nm. Calculate the caspase 3 activity in pmol of pNA released per min per μg recombinat human caspase 3.

Calculation

$$\text{Activity, pmol pNA/min/μg} = \frac{OD \times \mathrm{d}}{\varepsilon^{mM} \times t} / \text{amount of protein}$$

Where: $\varepsilon^{mM} = 10.5$

d - dilution factor

t - reaction time in minutes

The specific activity of recombinant human caspase 3 is 2196pmol/min/μg.

[IDENTIFICATION]

Figure 1. SDS-PAGE

Sample: Active recombinant CASP3, Human

[IMPORTANT NOTE]

The kit is designed for in vitro and research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.