

APA244Hu04 100µg

Active Caspase 2 (CASP2)

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: Prokaryotic expression.

Host: *E. coli*

Residues: Gly170~Thr452

Tags: N-terminal His-tag

Purity: >90%

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: 6.9

Predicted Molecular Mass: 35.4kDa

Accurate Molecular Mass: 18&12kDa 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.

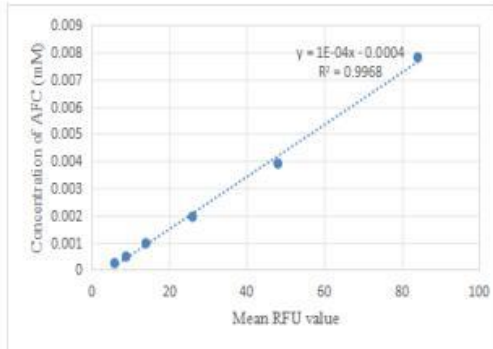
Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date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

[**SEQUENCE**]

```
GPVCLQVKPCTPEFYQTHFQLAYRLQSRPRGLALVLSNVHFTGKEKELEFRSGGDVDHSTLVTLFKLLGY
DVHVLCDQTAQEMQEKLQNF AQLPAHRVTDSCIVALLSHGVEGAIYGVDGKLLQLQEVFQLFDNANCP
LQNKPKMFFIQACRGDETDGRGVDQDQGNHAGSPGCEESDAGKEKLPKMRLPTRSDMICGYACLKGTAA
MRNTKRGSWYIEALAQVF SERACDMHVADMLVKVNALIKDREGYAPGTEFHRCKEMSEYCSSTLCRHLV
L
FPGHPT
```

[**ACTIVITY**]

Caspase-2 (CASP2) is a 30-32 kDa member of the peptidase C14A/IL-1 beta-converting family of enzymes. It is widely expressed and is an integral component of the apoptotic cascade. Based on the length of its prodomain, caspase-2 has been considered to be an initiator caspase. Human procaspase-2 is a 48-51 kDa, 452 amino acid (aa) protein. It is known to exist as a disulfide-linked homodimer via covalent linkage at Cys436. But this dimeric state may not be sufficient for (auto) activation. Actual activation may occur following oligomerization within the context of activating platforms such as DISC (death-inducing signaling complex) or the PIDDosome. The activity assay of recombinant human CASP2 was measured by its ability to cleave the fluorogenic peptide substrate Ac-VDVAD-AFC. The reaction was performed in 25 mM HEPES, 0.1% (w/v) CHAPS, 10 mM dithiothreitol (DTT), pH 7.5 (Assay Buffer). The CASP2 was diluted to 3 ug/ml by assay buffer and incubated at room temperature for 15min. The reaction was initiated by adding 50 ul 3 ug/ml CASP2 to 50 ul of 200 uM substrate and then read at excitation and emission wavelengths of 400 nm and 505 nm (top read), respectively, in kinetic mode for 5 minutes. The specific activity of recombinant human CASP2 is 2100 pmol/min/µg.



| RFU | AFC (mM) |
|----------|-------------|
| 84.11058 | 0.0078125 |
| 48.01058 | 0.00390625 |
| 25.99058 | 0.001953125 |
| 13.96058 | 0.000976563 |
| 8.83958 | 0.000488281 |
| 5.87658 | 0.000244141 |

Figure 1. The standard curve of AFC.

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

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

ΔRFU=Adjusted for Substrate Blank

F=Conversion Factor(convert from standard curve of AFC)

T= Time

N=Amount of enzyme

[IDENTIFICATION]

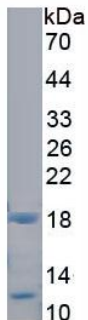


Figure 2. SDS-PAGE

Sample: Active recombinant CASP2, 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.