

APB058Hu01 100µg

Active Caspase Activated DNase (CAD)

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: Arg87~Lys323

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% Sarcosyl, 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: 8.4

Predicted Molecular Mass: 31.1kDa

Accurate Molecular Mass: 29kDa 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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                                     RFLS AFHEPQVGLI
QAAQQLLCDE QAPQRQLLA DLLHNVSQNI AAETRAEDPP WFEGLESRFQ
SKSGYLRYSK ESIRSYLRE VSSYPSTVGA EAQEEFLRVL GSMCQRLRSM
QYNGSYFDRG AKGGSRLCTP EGWFSCQGPF DMDSCLSRHS INPYSNRESR
ILFSTWNLDH IIEKKRTIIP TLVEAIKEQD GREVDWEYFY GLLFTSENLK
LVHIVCHKKT THKLNCDPSR IYK
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[ACTIVITY]

Caspase Activated DNase (CAD), corresponding to product code RPB058Hu01, is a key nuclease in eukaryotic apoptosis. Normally, it exists in an inactive state in the cytoplasm, bound to its inhibitor. Upon apoptotic signal activation, effector caspases (e.g., Caspase-3) cleave the inhibitor, releasing active CAD. The activated enzyme translocates to the nucleus, where it cleaves chromosomal DNA into nucleosome-sized fragments, a hallmark of apoptotic cell death. Its strict regulation prevents unintended DNA damage, ensuring genomic stability in healthy cells. CAD interacts with DFFa (product code L426Hu) as its specific inhibitor; DFFa binds CAD to inhibit its nuclease activity in non-apoptotic cells. Thus a functional ELISA assay was conducted to detect the interaction of recombinant human CAD and recombinant human DFFa. Briefly, CAD was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 μ l were then transferred to DFFa-coated microtiter wells and incubated for 1h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-CAD pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37 °C, wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 °C. Finally, add 50 μ L stop

solution to the wells and read at 450/630nm immediately. The binding activity of recombinant human CAD and recombinant human DFFa was shown in Figure 1, the EC50 for this effect is 0.01067µg/mL.

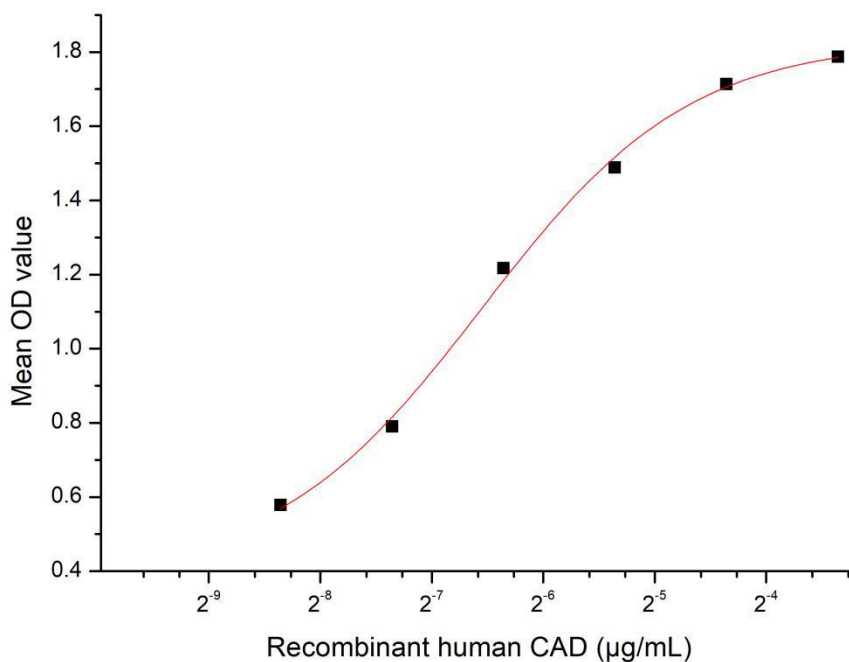


Figure 1. The binding activity of recombinant human CAD and human DFFa

[IDENTIFICATION]

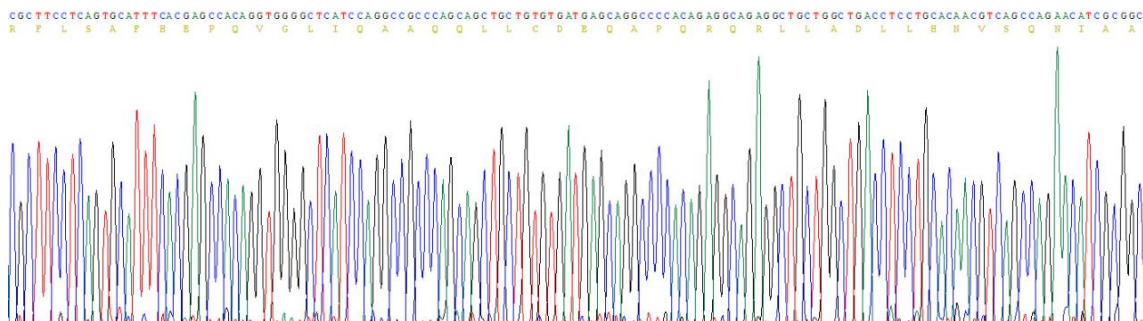


Figure 2. Gene Sequencing (extract)

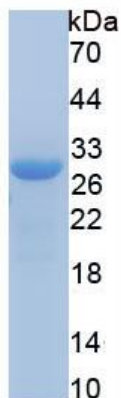


Figure 3. SDS-PAGE

Sample: Active recombinant CAD, 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.