APC457Ra01 10μg Active Endonuclease G, Mitochondrial (ENDOG) Organism Species: *Rattus norvegicus (Rat) 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: Met1~Lys294 Tags: N-terminal His and GST 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: 350µg/mL Applications: Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 9.8 Predicted Molecular Mass: 62.3kDa Accurate Molecular Mass: 62kDa as determined by SDS-PAGE reducing conditions.

## [<u>USAGE</u>]

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.

# Cloud-Clone Corp.

**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]

MRALRAGLTL ALGAGLGAAA EHWRRREGKG PGLLGRVPVL PVVAADLPAL PGGPAGSTGE LAKYGLPGVA QLRSRESYVL SYDPRTRGAL WVLEQLRPER LRGDGDRRAC DFHEDDSVHA YHRATNADYR GSGFDRGHLA AAANHRWSQR AMDDTFYLSN VAPQVPHLNQ HAWNNLEKYS RSLTRTYQNV YVCTGPLFLP RTEADGKSYV KYQVIGKNHV AVPTHFFKVL ILEAASGQIE LRSYVMPNAP VDETLPLERF LVPIESIERA SGLLFVPNIL ARAGNLKAIT AGSK

## [ACTIVITY]

Endonuclease G, Mitochondrial (ENDOG) is a conserved nuclease encoded by nuclear DNA and localized in the mitochondrial intermembrane space. It plays dual roles in apoptosis and mitochondrial genome maintenance. During programmed cell death, ENDOG is released into the cytosol, cleaving nuclear and mitochondrial DNA at specific sites to facilitate apoptotic degradation. Structurally, it functions as a homodimer with Mg<sup>2</sup> + -dependent endonuclease activity. Beyond apoptosis, ENDOG participates in mitochondrial DNA replication, repair, and maternal genome processing during embryogenesis. Notably, ENDOG interacts with cytochrome c (CYCS) to synergistically amplify apoptosis. This complex enhances nucleolytic DNA degradation and modulates the mitochondrial permeability transition pore (MPTP), further promoting cell death.Thus a functional ELISA assay was conducted to detect the interaction of recombinant rat ENDOG and recombinant human CYCS. Briefly, ENDOG was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100  $\mu$  I were then transferred to CYCS-coated microtiter wells and incubated for 1h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-ENDOG 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.

# Cloud-Clone Corp.

With the addition of substrate solution, wells were incubated 15-25 minutes at 37  $^\circ C$ . Finally, add 50  $\mu L$  stop solution to the wells and read at 450/630nm immediately. The binding activity of recombinant rat ENDOG and recombinant human CYCS was shown in Figure 1, the EC50 for this effect is 1.01ug/mL.



Figure 1. The binding activity of recombinant rat ENDOG and recombinant human CYCS

# Cloud-Clone Corp.

# [IDENTIFICATION]



Figure 2. Gene Sequencing (extract)



Figure 3. SDS-PAGE

Sample: Active recombinant ENDOG, 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.