APC457Ra01 100µg Active Endonuclease G, Mitochondrial (ENDOG) Organism Species: Homo sapiens (Human) *Instruction manual*

FOR IN VITRO USE AND RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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1th Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: E. coli

Residues: Met1~Lys294

Tags: Two N-terminal Tags, His-tag and GST-tag

Purity: >92%

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 5% trehalose.

Applications: Cell culture; Activity Assays; In vivo assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 9.5

Predicted Molecular Mass: 62.3kDa

Accurate Molecular Mass: 62kDa as determined by SDS-PAGE reducing conditions.

[<u>USAGE</u>]

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.

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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.

[<u>SEQUENCE</u>]

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]

ENDOG (Endonuclease G) is a mitochondrial enzyme that cleaves DNA at double-stranded (DG)n. (DC)n and at single-stranded (DC)n tracts. This protein also has ribonuclease (RNase) and RNase H activities. It has been reported that EndoG forms complexes with some other proteins, including HSP70 (Heat shock 70 kDa protein). Besides, mouse HSPA1A (Heat shock 70 kDa protein 1A) shares similarities with rat HSP1A1 in amino acid sequence with the identity of 98.44%. Thus, a binding ELISA assay was conducted to detect the association of recombinant rat ENDOG with recombinant mouse HSPA1A. Briefly, ENDOG were diluted serially in PBS with 0.01%BSA (pH 7.4). Duplicate samples of 100uL were then transferred to HSP1A1-coated microtiter wells and incubated for 2h 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, wells were incubated 15-25 minutes at 37°C. Finally, add 50µL stop solution to the wells and read at 450nm immediately. The binding activity of

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ENDOG with HSP1A1 was shown in Figure 1 and this effect was in a dose dependent manner.

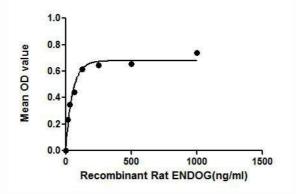


Figure 1. The binding activity of ENDOG with HSP1A1

[IDENTIFICATION]

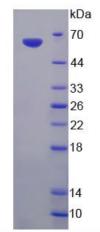


Figure 2. SDS-PAGE

Sample: Active recombinant ENDOG, Human



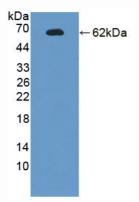


Figure 3. Western Blot

Sample: Recombinant ENDOG, Human;

Antibody: Rabbit Anti-Human ENDOG Ab (PAC457Ra01)

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