

APL997Hu01 100µg

Active Protein L-Isoaspartate-O-Methyltransferase (PCMT1)

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: Ala2~Lys227

Tags: N-terminal His-tag

Purity: >95%

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: Cell culture; Activity Assays.

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

Predicted isoelectric point: 6.8

Predicted Molecular Mass: 28.2kDa

Accurate Molecular Mass: 28kDa 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]

AWKSGGASH SELIHNLRKN GIIKTDKVFE VMLATDRSHY AKCNPYMDSP
QSIGFQATIS APHMAYALE LLFDQLHEGA KALDVSGSGS ILTACFARMV
GCTGKVIGID HIKELVDDSV NNRKDDPTL LSSGRVQLVV GDGRMGYAAE
APYDAIHVGA AAPVVPQALI DQLKPGGRLI LPVGPAGGNQ MLEQYDKLQD
GSIKMKPLMG VIYVPLTDKE KQWSRWK

[ACTIVITY]

Protein L-Isoaspartate-O-Methyltransferase (PCMT1) is a crucial enzyme involved in the repair of damaged proteins. It catalyzes the methylation of abnormal L-isoaspartyl residues and, to a lesser extent, D-aspartyl residues in proteins, which arise from the spontaneous deamidation and isomerization of asparagine and aspartate residues over time. This post-translational modification serves as a key step in the repair process, allowing these damaged proteins to be either restored to their normal structure or targeted for degradation, thereby maintaining protein homeostasis within cells. As PCMT1 has the function of cell proliferation, we measure the activity of recombinant human PCMT1 by the ability of the protein to stimulate MCF-7 cells proliferation. MCF-7 cells were seeded into triplicate wells of 96-well plates and allowed to attach, replaced with various concentrations of recombinant human PCMT1. After incubated for 72h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10 µl of CCK-8 solution was added to each well of the plate, then the absorbance at 450 nm was measured using a microplate reader after incubating the plate for 1-4 hours at 37 °C. Cell viability was assessed by CCK-8 assay after incubation with recombinant human PCMT1 for 72h. The result was shown in Figure 1. It was obvious that PCMT1 significantly increased cell viability

of MCF-7 cells. The ED₅₀ of recombinant human PCMT1 is 2.059 μ g/ml.

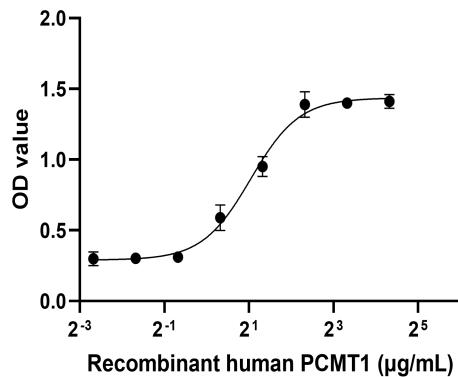


Figure 1 The dose-effect curve of recombinant human PCMT1 on MCF-7 cells

[IDENTIFICATION]

GAATTCGCCCTGGAAATCCGGCGGCCAGCCACTCGGAGCTAATCCACAACTCTCCGCAAAAATGGAATCATCAAGACAGATAAAAGTATTGAAAGTGATGCTGGCTACAGACCGCTCCCACTATGCAAAATGTAACCCATACATGGATTCTCCACAAT

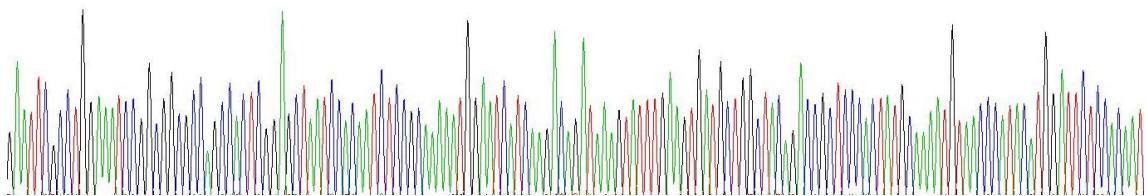


Figure 2. Gene Sequencing (extract)

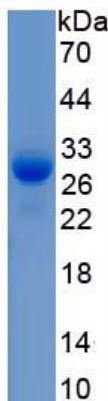


Figure 3. SDS-PAGE

Sample: Active recombinant PCMT1, 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.