

APC744Hu01 100µg

Active Peptidylglycine Alpha Amidating Monooxygenase (PAM)

Organism Species: Homo sapiens (Human)

Instruction manual

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

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Phe21~Cys288

Tags: N-terminal His-tag

Purity: >95%

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

Applications: Cell culture; Activity Assays.

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

Predicted isoelectric point: 8.8

Predicted Molecular Mass: 33.7kDa

Accurate Molecular Mass: 34kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

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.

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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FRSPLSVFKR FKETTRPFSN ECLGTTTRPVV
PIDSSDFALD IRMPGVTPKQ SDTYFCMSMR IPVDEEAFVI DFKPRASMDT
VHHMLLFGCN MPSSTGSYWF CDEGTCTDKA NILYAWARNA PPTRLPKGVG
FRVGGETGSK YFVLQVHYGD ISAFRDNNKD CSGVSLHLTR LPQPLIAGMY
LMMSVDTVIP AGEKVVNSDI SCHYKNYPMH VFAYRVHTHH LGKVVSGYRV
RNGQWTLIGR QSPQLPQAFY PVGHPVDVSF GDLLAARC
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[ACTIVITY]

Peptidyl-glycine alpha-amidating monooxygenase (PAM) is an enzyme that is required for the biosynthesis of many signaling peptides. This enzyme mainly includes two domains with distinct catalytic activities, a peptidylglycine alpha-hydroxylating monooxygenase (PHM) domain and a peptidyl-alpha-hydroxyglycine alpha-amidating lyase (PAL) domain. These catalytic domains work sequentially to catalyze neuroendocrine peptides to active alpha-amidated products. Besides, Glucosidase Alpha, Acid (GaA) has been identified as an interactor of PAM, thus a binding ELISA assay was conducted to detect the interaction of recombinant human PAM and recombinant human GaA. Briefly, PAM were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to GaA-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-PAM pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, 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 PAM and GaA.was shown in Figure 1, and this effect was in a dose dependent manner.

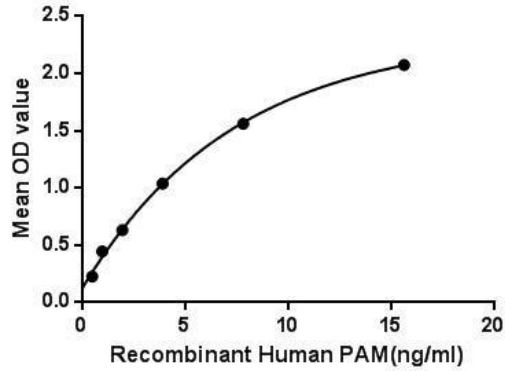


Figure 1. The binding activity of PAM with GaA.

[IDENTIFICATION]

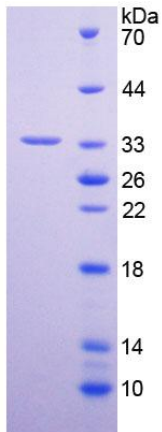


Figure 2. SDS-PAGE

Sample: Active recombinant PAM, Human

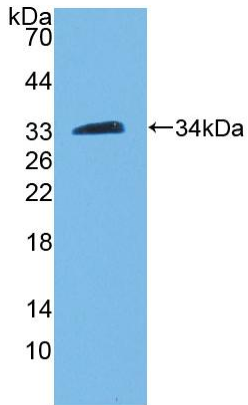


Figure 3. Western Blot

Sample: Recombinant PAM, Human;

Antibody: Rabbit Anti-Human PAM Ab (PAC744Hu01)