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

1th 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^{o}C for one month.
Aliquot and store at -80^{o}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 37ºC 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]

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

[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℃. 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℃. 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.

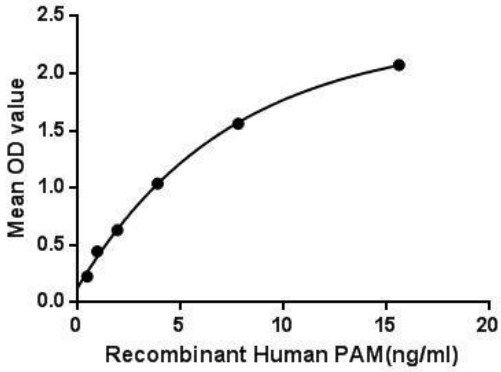

Figure 1. The binding activity of PAM with GaA.

[IDENTIFICATION]

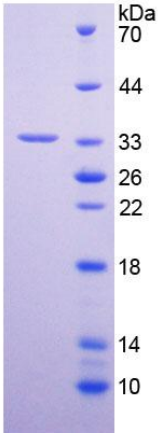

Figure 2. SDS-PAGE

Sample: Active recombinant PAM, Human

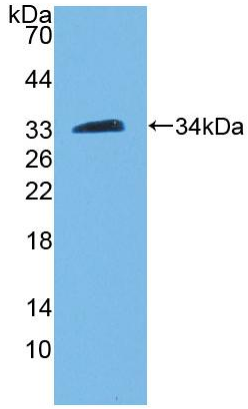

Figure 3. Western Blot

Sample: Recombinant PAM, Human;

Antibody: Rabbit Anti-Human PAM Ab (PAC744Hu01)