APC744Hu61 100μg
Active Peptidylglycine Alpha Amidating Monooxygenase (PAM)
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: Eukaryotic expression.
Host: 293F cell
Residues: Phe31~Val817
Tags: N-terminal His-tag
Purity: >90%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 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: 6.1
Predicted Molecular Mass: 89.7kDa
Accurate Molecular Mass: 70kDa as determined by SDS-PAGE reducing conditions.
Phenomenon explanation:
The possible reasons that the actual band size differs from the predicted are as follows:
1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

[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]

FKETTRPFSNECLGTTRPVVPIDSSDFALDIRMPGVTPKQSDTYFCMSMRIPVDEEAFVIDFKPRASMDTVHHMLLFGCNMPSSTGSY
WFCDEGTCTDKANILYAWARNAPPTRLPKGVGFRVGGETGSKYFVLQVHYGDISAFRDNNKDCSGVSLHLTRLPQPLIAGMYLMMSVD
TVIPAGEKVVNSDISCHYKNYPMHVFAYRVHTHHLGKVVSGYRVRNGQWTLIGRQSPQLPQAFYPVGHPVDVSFGDLLAARCVFTGEG
RTEATHIGGTSSDEMCNLYIMYYMEAKHAVSFMTCTQNVAPDMFRTIPPEANIPIPVKSDMVMMHEHHKETEYKDKIPLLQQPKREEE
EVLDQGDFYSLLSKLLGEREDVVHVHKYNPTEKAESESDLVAEIANVVQKKDLGRSDAREGAEHERGNAILVRDRIHKFHRLVSTLRP
PESRVFSLQQPPPGEGTWEPEHTGDFHMEEALDWPGVYLLPGQVSGVALDPKNNLVIFHRGDHVWDGNSFDSKFVYQQIGLGPIEEDT
ILVIDPNNAAVLQSSGKNLFYLPHGLSIDKDGNYWVTDVALHQVFKLDPNNKEGPVLILGRSMQPGSDQNHFCQPTDVAVDPGTGAIY
VSDGYCNSRIVQFSPSGKFITQWGEESSGSSPLPGQFTVPHSLALVPLLGQLCVADRENGRIQCFKTDTKEFVREIKHSSFGRNVFAI
SYIPGLLFAVNGKPHFGDQEPVQGFVMNFSNGEIIDIFKPVRKHFDMPHDIVASEDGTVYIGDAHTNTVWKFTLTEKLEHRSV

[ACTIVITY]

Peptidyl-glycine alpha-amidating monooxygenase (PAM) is an enzyme that is required for the biosynthesis of many signaling peptides. It has two enzymatically active domains with catalytic activities-peptidylglycine alpha-hydroxylating monooxygenase (PHM) and peptidyl-alpha-hydroxyglycine alpha-amidating lyase (PAL). These catalytic domains work sequentially to catalyze neuroendocrine peptides to active alpha-amidated products. A typical activity assay was using Dns-Tyr-Val-Gly as substrate, thus the activity of recombinant human PAM was measured by its ability to hydrolyze Dns-Tyr-Val-Gly to Dns-Tyr-Val-NH2. The reaction was performed in 100 mM MES/KOH pH 6.0, 30 mM KI, 30 mM KCl, 1 uM

cupric sulfate, 100 ug/ml catalase (APC418Hu05), 1% (v/v) ethanol, 0.001% (v/v) Triton X-100 and 10 mM ascorbate. 250 ul 0.7 mM substrate of Dns-Tyr-Val-Gly was added with 250 ul various concentrations of recombinant human PAM (1 ug/ml,5 ug/ml, 10 ug/ml and 20 ug/ml). Incubated at 37℃ for 30min, the reaction was stopped by addition 6% (v/v) TCA. The product and substrate was detected by RP-HPLC with UV-detection at 280 nm, the analyses were performed at 25℃ employing a Agilent ZORBAX Poroshell SB C18 column (9.4×250 mm, 5 μm), the flow rate was 1 ml/min. The mobile phase consisted of 100 mM sodium acetate (pH 6.5) and 30 min linear gradient of 10-90% acetonitrile. At 30-35 min, the mobile phase consisted of 90% acetonitrile and 10% sodium acetate. The result was shown in Figure 1. As the Figure 1 shows, the substrate have been hydrolyzed to Dns-Tyr-Val-NH2 after incubating with recombinant human PAM. The retention time of Dns-Tyr-Val-Gly and Dns-Tyr-Val-NH2 is 24.088 and 30.421 respectively (Figure 2). The specific activity of recombinant human PAM is >6600 pmol/min/μg.

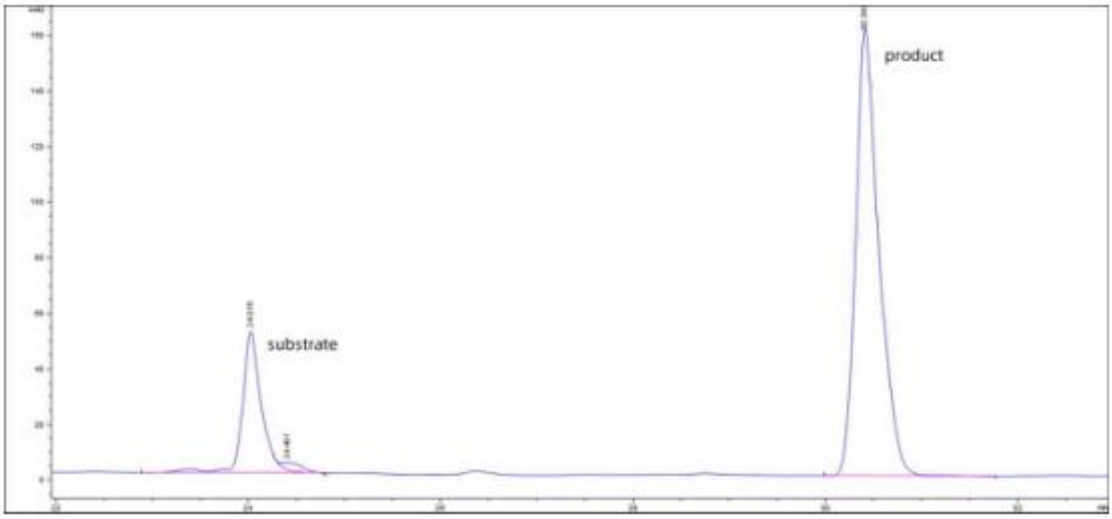


Figure 1. Recombinant human PAM activity assay by HPLC

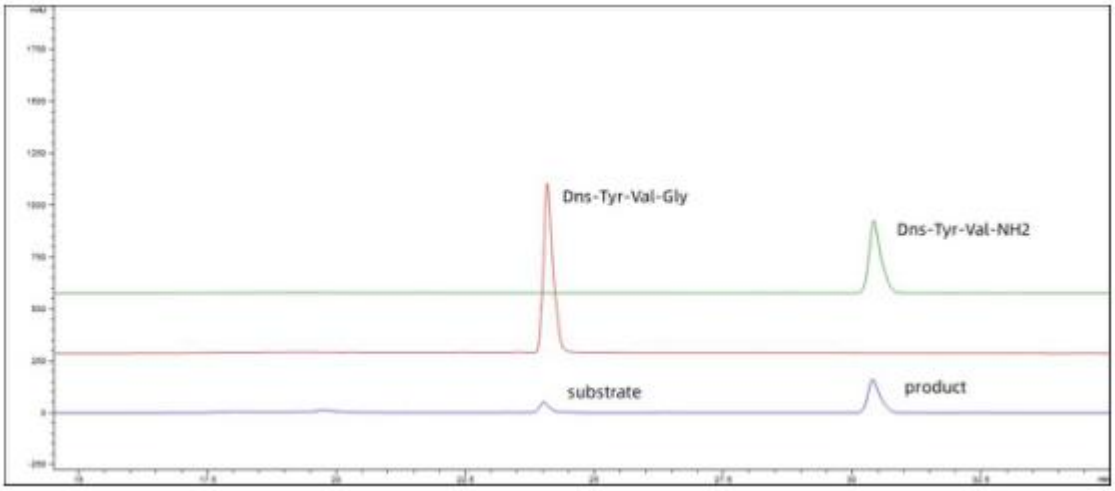
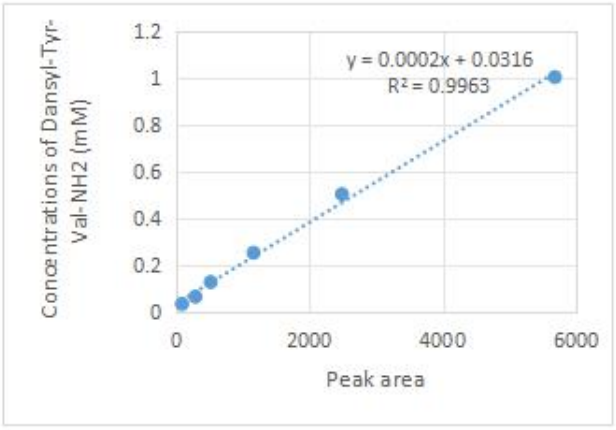


Figure 2. The reaction product compared with standard Dns-Tyr-Val-Gly and Dns-Tyr-Val-NH_2.



PEAK AREA	DNS-Tyr-Val-NH2 (mM)
5683.9	1
2490.9	0.5
1166.7	0.25
522.1	0.125
294.7	0.0625
93.4	0.03125

Figure 3. The sandard curve of Dns-Tyr-Val-NH_2

[IDENTIFICATION]

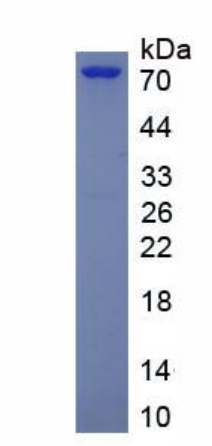


Figure 4. SDS-PAGE

Sample: Active recombinant PAM, 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.