APC744Ra61 100μg
Active Peptidylglycine Alpha Amidating Monooxygenase (PAM)
Organism Species: *Rattus norvegicus* (Rat)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Eukaryotic expression.
Host: 293F cell
Residues: Phe36~Val715
Tags: N-terminal His-tag
Purity: >96%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: 10mM PBS, pH7.6, containing 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: 5.7
Predicted Molecular Mass: 77.7kDa
Accurate Molecular Mass: 88kDa 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 (pH7.6) 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]

FKETT RSFSNECLGT
IGPVTPLDAS DFALDIRMPG VTPKESDTYF CMSMRLPVDE EAFVIDFKPR
ASMDTVHHML LFGCNMPSST GSYWFCDEGT CTDKANILYA WARNAPPTRL
PKGVGFRVGG ETGSKYFVLQ VHYGDISAFR DNHKDCSGVS VHLTRVPQPL
IAGMYLMMSV DTVIPPGEKV VNADISCQYK MYPMHVFAYR VHTHHLGKVV
SGYRVRNGQW TLIGRQNPQL PQAFYPVEHP VDVTFGDILA ARCVFTGEGR
TEATHIGGTS SDEMCNLYIM YYMEAKYALS FMTCTKNVAP DMFRTIPAEA
NIPIPVKPDM VMMHGHHKEA ENKEKSALMQ QPKQGEEEVL EQDFHVEEEL
DWPGVYLLPG QVSGVALDSK NNLVIFHRGD HVWDGNSFDS KFVYQQRGLG
PIEEDTILVI DPNNAEILQS SGKNLFYLPH GLSIDTDGNY WVTDVALHQV
FKLDPHSKEG PLLILGRSMQ PGSDQNHFCQ PTDVAVEPST GAVFVSDGYC
NSRIVQFSPS GKFVTQWGEE SSGSSPRPGQ FSVPHSLALV PHLDQLCVAD
RENGRIQCFK TDTKEFVREI KHASFGRNVF AISYIPGFLF AVNGKPYFGD
QEPVQGFVMN FSSGEIIDVF KPVRKHFDMP HDIVASEDGT VYIGDAHTNT
VWKFTLTEKM EHRSV

[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 using Dns-Tyr-Val-Gly as substrate, thus the recombinant rat PAM activity was measured by its ability to hydrolyze Dns-Tyr-Val-Gly to Dns-Tyr-Val-NH2. The reaction was preformed in 1mL containing 100mM MES/KOH pH 6.0, 30mM KI, 30mM KCl, 1μmol/L cupric sulfate, 100ug/mL catalase, 1% (v/v) ethanol, 0.001% (v/v) Triton X-100, 10mM ascorbate, 0.35mM/L Dns-Tyr-Val-Gly (0.2mg/mL) and initiated by addition various concentrations of PAM (0.1ug/mL, 1ug/mL, 5ug/mL). Incubated at 37℃ for 30min, the reaction stopped by addition 6% (v/v) TFA. The product and substrate was detected by RP-HPLC with UV-detection at 280nm, the analyses were performed at 25℃ employing a Agilent ZORBAX Poroshell SB C18 column (9.4×250mm, 5μm), the flow rate was 1ml/min. The mobile phase consisited of 100 mM sodium acetate (pH 6.5) and 35min linear gradient of 10-90% acetonitrile. The result was shown in Figure 1.

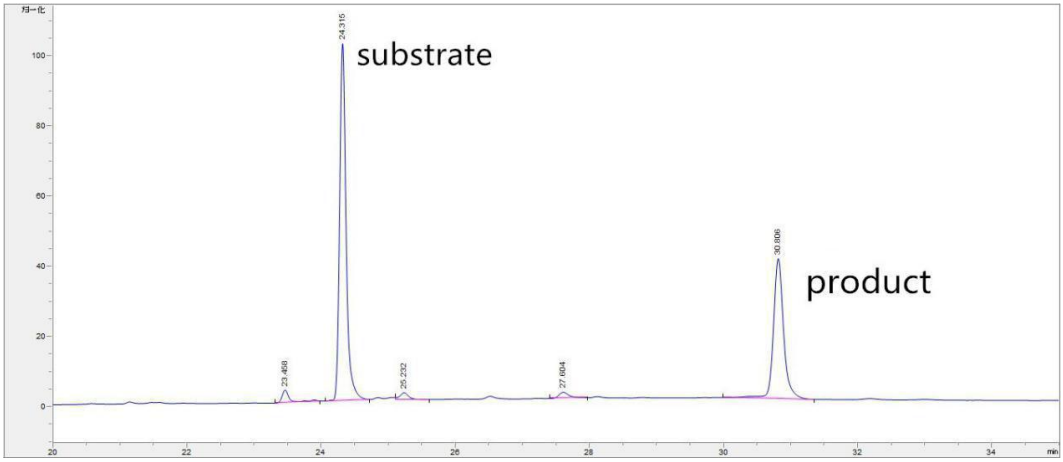


Figure 1. Recombinant Rat PAM activity assay by HPLC.

As the Figure 1 shows, after 30min later ,the substrate have been hydrolyzed when the PAM was 5ug/mL. The retention time of Dns-Tyr-Val-Gly and Dns-Tyr-Val-NH2 is 24.315 and 30.806 respectively.

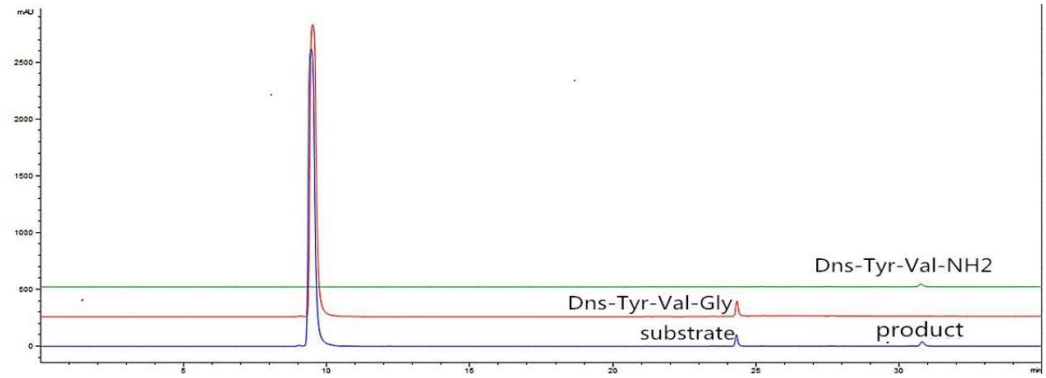
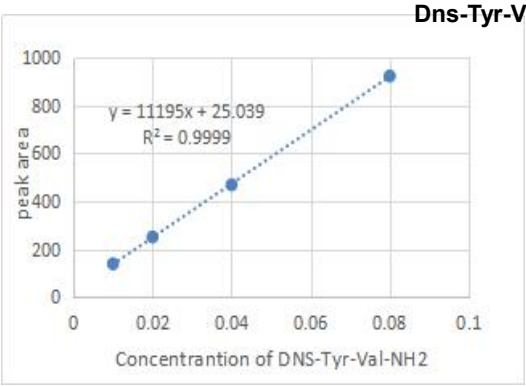


Figure 2. The reaction product compared with standard Dns-Tyr-Val-Gly and Dns-Tyr-Val-NH2.



DNS-Tyr-Val-NH2 (mg/mL)	PEAK AREA
0.01	138.3
0.02	250.5
0.04	468.2
0.08	922.4

Figure 3. The sandard curve of Dns-Tyr-Val-NH2.

One unit of enzyme activity is defined as the amount of enzyme required to convert 1μmol of substrate to amidated product in 1min at 37°C. Thus the recombinant rat PAM activity is 2.4×10^6U.

[IDENTIFICATION]

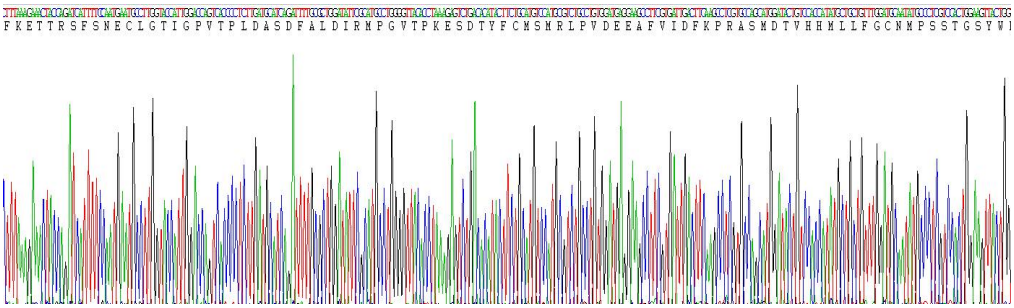


Figure 4. Gene Sequencing (extract)

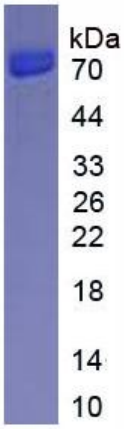


Figure 5. SDS-PAGE

Sample: Active recombinant PAM, Rat

[IMPORTANT NOTE]

The kit is designed for in vitro and research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.