

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

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Eukaryotic expression.

Host: 293F cell

Residues: Phe36~Val820

Tags: N-terminal His-tag

Purity: >96%

Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

Buffer Formulation: PBS, pH7.4, containing 5% Trehalose.

Original Concentration: 100µg/mL

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: 89.5kDa

Accurate Molecular Mass: 88kDa 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]

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FKETTRSF SNECLGTIGPVTPLDASDFALDIRMPGVTPKESDITYFCMSMRLPVDEEAFVIDFKPRASMD
TVHHMLLFGCNMPSSSTGSYWFCDDEGTCTDKANILYAWARNAPPTRLPKGVGFRVGGGETGSKYFVLQVHY
GDISAFRDNHKDCSGVSVHLTRVPQPLIAGMYLMM SVDTVI PPGEKVVNADI SCQYKMYPMHVFA YRVH
THHLGKVVSGYRVRNGQWTLIGRQNPQLPQAFYPVEHPVDVTFGDILAARCVFTGEGRTEATHIGGTSS
DEM CNLYIMYYMEAKYALSFMTCTKNVAPDMFRTIPAEANIPIPVKPDMVMMHGHKKEAENKEKSALMQ
QPKQGEVVLEQGDFFYSLLSKLLGEREDVHVHKYNPTEKTESGSDLVAEIANVVQKKDLGRSDAREGAE
HEEWGNAILVRDRIHRFHQLESTLRPAESRAF SFQQPGEGPWEPEPSGDFHVEEELDWPGVYLLPGQVS
GVALDSKNNLVI FHRGDHVWDGNSFDSKFVYQQRGLGPIEEDTILVIDPNNAEILQSSGKNLFYLPHGL
SIDTDGNYWVTDVALHQVFKLDPHSKEGPLLILGRSMQPGSDQNHFCQPTDVAVEPSTGAVFVSDGYCN
SRI VQFSPSGKFVTQWGEESGSSPRPGQF SVPHSLALVPHLDQLCVADRENGRIQCFKTDTKEFVREI
KHASFGRNVFAISYIPGFLFAVNGKPYFGDQEPVQGFVMMNFSSGEIIDVFKPVRKHFDMPHDIVASEDG
TVYIGDAHTNTVWKFTLTEKMEHRVS
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[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 performed 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°C for 30min, the reaction stopped by addition 6% (v/v) TCA. The product and substrate was detected by RP-HPLC with UV-detection at 280nm, the analyses were performed at 25°C employing a Agilent ZORBAX Poroshell SB C18 column (9.4×250mm, 5µm), the flow rate was 1ml/min. The mobile phase consisted of 100 mM sodium acetate (pH 6.5) and

35min linear gradient of 10-90% acetonitrile. The result was shown in Figure 1. As the Figure 1 shows, after 30min later, the substrate have been hydrolyzed when the PAM was 5 ug/ml. The retention time of Dns-Tyr-Val-Gly and Dns-Tyr-Val-NH₂ is 24.315 and 30.806 respectively. The specific activity of recombinant rat PAM is > 7600 pmol/min/μg.

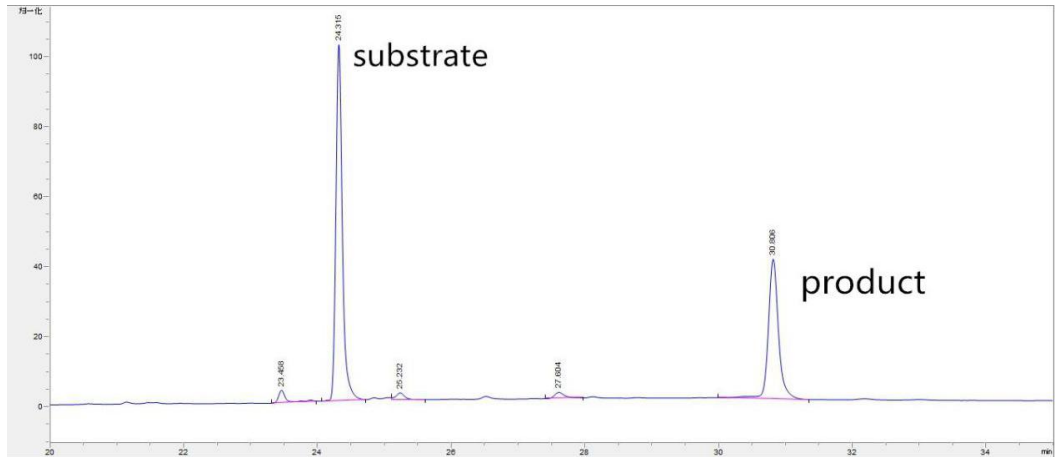


Figure 1. Recombinant Rat PAM activity assay by HPLC

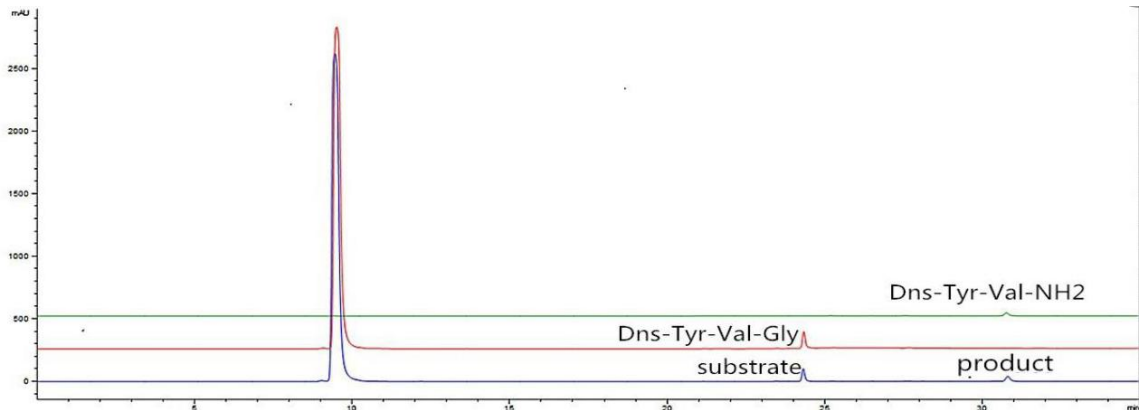


Figure 2. The reaction product compared with standard Dns-Tyr-Val-Gly and Dns-Tyr-Val-NH₂.

