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: >90%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 5% Trehalose.
Original Concentration: 250μg/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]

FKETTRSFSNECLGTIGPVTPLDASDFALDIRMPGVTPKESDTYFCMSMRLPVDEEAFVIDFKPRASMD
TVHHMLLFGCNMPSSTGSYWFCDEGTCTDKANILYAWARNAPPTRLPKGVGFRVGGETGSKYFVLQVHY
GDISAFRDNHKDCSGVSVHLTRVPQPLIAGMYLMMSVDTVIPPGEKVVNADISCQYKMYPMHVFAYRVH
THHLGKVVSGYRVRNGQWTLIGRQNPQLPQAFYPVEHPVDVTFGDILAARCVFTGEGRTEATHIGGTSS
DEMCNLYIMYYMEAKYALSFMTCTKNVAPDMFRTIPAEANIPIPVKPDMVMMHGHHKEAENKEKSALMQ
QPKQGEEEVLEQGDFYSLLSKLLGEREDVHVHKYNPTEKTESGSDLVAEIANVVQKKDLGRSDAREGAE
HEEWGNAILVRDRIHRFHQLESTLRPAESRAFSFQQPGEGPWEPEPSGDFHVEEELDWPGVYLLPGQVS
GVALDSKNNLVIFHRGDHVWDGNSFDSKFVYQQRGLGPIEEDTILVIDPNNAEILQSSGKNLFYLPHGL
SIDTDGNYWVTDVALHQVFKLDPHSKEGPLLILGRSMQPGSDQNHFCQPTDVAVEPSTGAVFVSDGYCN
SRIVQFSPSGKFVTQWGEESSGSSPRPGQFSVPHSLALVPHLDQLCVADRENGRIQCFKTDTKEFVREI
KHASFGRNVFAISYIPGFLFAVNGKPYFGDQEPVQGFVMNFSSGEIIDVFKPVRKHFDMPHDIVASEDG
TVYIGDAHTNTVWKFTLTEKMEHRSV

[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℃ 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℃ 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. 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.

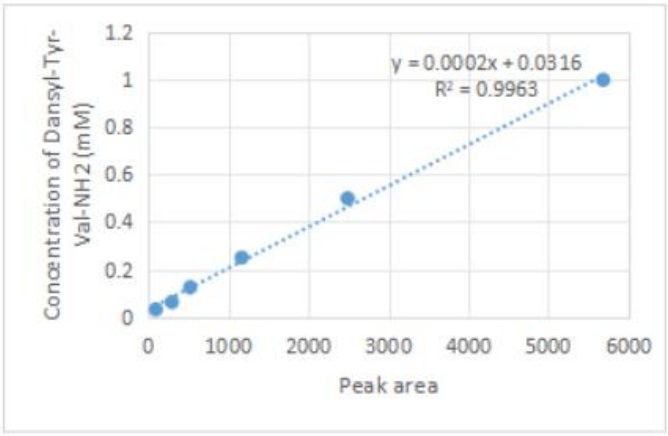

Figure 1. The standard curve of Dns-Tyr-Val-NH_2

Peak area	Dansyl-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

Specific Activity (pmol/min/μg) =

$$\frac{\text{Adjusted Vmax * (peak area/min) x Conversion Factor ** (pmol/peak area)}}{\text{amount of enzyme (ug)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard Dns-Tyr-Val-NH2

[IDENTIFICATION]

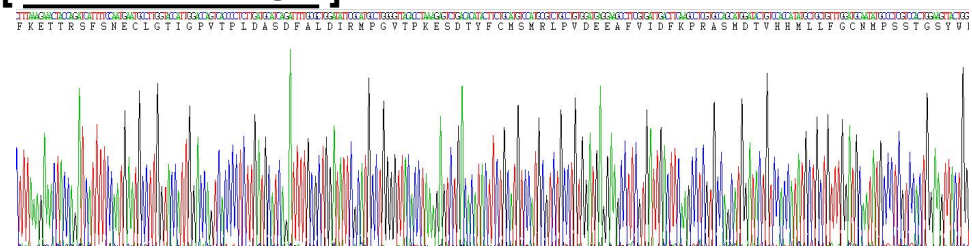

Figure 2. Gene Sequencing (extract)

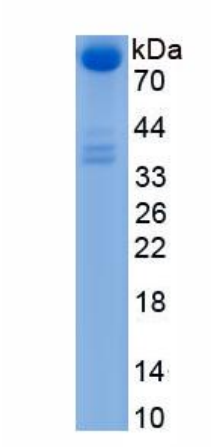

Figure 3. SDS-PAGE

Sample: Active recombinant PAM, Rat

[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.