

Peptidylglycine Alpha Amidating Monooxygenase (PAM) Active Protein | PAM active protein

Active Peptidylglycine Alpha Amidating Monooxygenase (PAM)

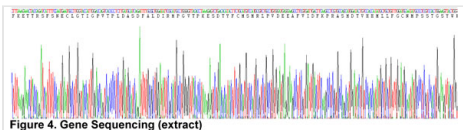
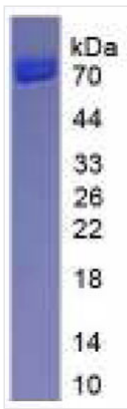
CATALOG # AAA21144	HOST 293F cell	APPLICATIONS Cell Culture, Activity Assay
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PRODUCT SYNONYMS

Peptidylglycine Alpha Amidating Monooxygenase (PAM); Active Peptidylglycine Alpha Amidating Monooxygenase (PAM); PAM active protein

PRODUCT SPECIFICATIONS

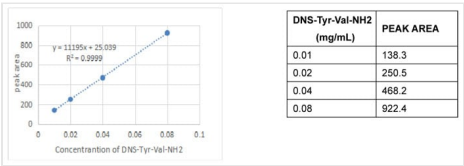
Host	293F cell
Purity/Purification	>95%
Form/Format	PBS, pH7.4, containing 5% Trehalose
Concentration	Original Concentration: 400ug/mL (varies by lot)
Applicable Applications for PAM active protein	Cell Culture, Activity Assay
Application Notes	(May be suitable for use in other assays to be determined by the end user)
Source	Eukaryotic expression
Residues	Phe36~Val820
Tags	N-terminal His-tag
Endotoxin Level	<1.0EU per 1ug (determined by the LAL method)
Predicted isoelectric point	5.7
Usage	Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex
Preparation and Storage	Storage: Avoid repeated freeze/thaw cycles. Store at 2-8 degree C for one month. Aliquot and store at -80 degree 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 degree 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.



Gene Sequencing (extract)

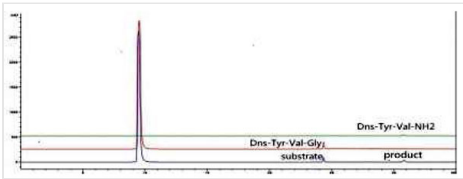
SDS-PAGE

Sample: Active recombinant PAM, Rat



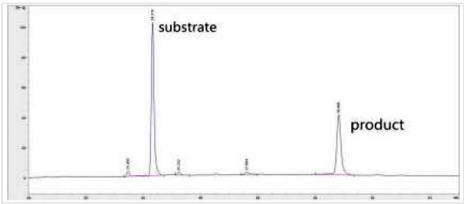
Application Data

Figure 3. The standard curve of Dns-Tyr-Val-NH2. One unit of enzyme activity is defined as the amount of enzyme required to convert 1umol of substrate to amidated product in 1min at 37°C. Thus the recombinant rat PAM activity is 2.4×106U.



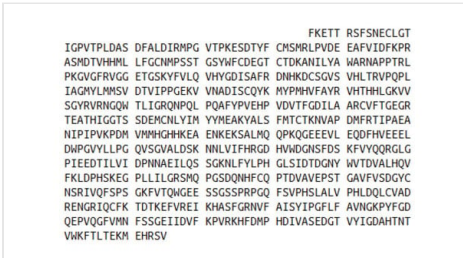
Application Data

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



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, 1umol/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 degree C 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 degree C employing a Agilent ZORBAX Poroshell SB C18 column (9.4×250mm, 5um), 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. 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.



Sequence Information

ADDITIONAL INFORMATION

Product Categories/Family for PAM active protein	Enzyme & Kinase
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NCBI AND UNIPROT INFORMATION

NCBI GI #	19923674
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NCBI GeneID	25508
NCBI GenBank Nucleotide #	NM_013000.2
Molecular Weight	Predicted Molecular Mass: 89.5kDa Accurate Molecular Mass: 88kDa as determined by SDS-PAGE reducing conditions.
NCBI Official Full Name	peptidyl-glycine alpha-amidating monooxygenase
NCBI Official Synonym Full Names	peptidylglycine alpha-amidating monooxygenase
NCBI Official Symbol	Pam
NCBI Official Synonym Symbols	PHM
NCBI Protein Information	peptidyl-glycine alpha-amidating monooxygenase
UniProt Protein Name	Peptidyl-glycine alpha-amidating monooxygenase
UniProt Gene Name	Pam
UniProt Synonym Gene Names	PAM; PHM; PAL
UniProt Entry Name	AMD_RAT

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Small volumes of product may become entrapped in the seal of the vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the cap.

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Online datasheet: <https://www.aaabiotech.com/293f-active-protein/peptidylglycine-alpha-amidating-monooxygenase-pam/21144>