Active Peptidylglycine Alpha Amidating Monooxygenase (PAM)

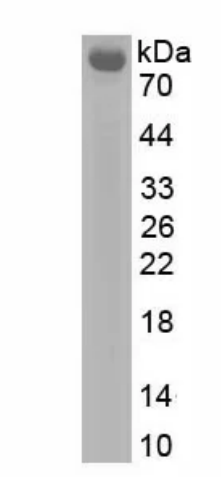
Catalog#:PHH12861

概述（Summary）

别称（Alternative Names）	PAL; PHM; Peptidylamidoglycolate lyase; Peptidyl-Alpha-hydroxyglycine Alpha-amidating Lyase; Peptidylglycine Alpha-Hydroxylating Monooxygenase
纯度（Purity）	Greater than 90% as determined by reducing SDS-PAGE.
内毒素（Endotoxin level）	Please contact with the lab for this information
蛋白构建（Construction）	【Phe31-Val817】
Accession #	P19021
表达宿主（Host）	293F
种属（Species）	Homo sapiens (Human)
预测分子量（Predicted MW）	89.7 kDa
制剂（Form）	Lyophilized from a 0.2 μm filtered solution of PBS, pH7.4, containing 0.01% SKL, 1mM DTT, 5% Trehalose and Proclin300.
运输方式（Shipping）	The product is shipped with ice bag. Upon receipt, store it immediately at the temperature listed below.
稳定性&储存（Stability &Storage）	12 months at -20°C to -80°C in lyophilized state.6 months at -20°C to -80°C under sterile conditions after reconstitution.7-10 days at 2°C to 8°C under sterile conditions after reconstitution.Please minimize freeze-thaw cycles.
复溶（Reconstitution）	Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.
背景介绍（References）	Bifunctional enzyme that catalyzes the post-translational modification of inactive peptidylglycine precursors to the corresponding bioactive alpha-amidated peptides, a terminal modification in biosynthesis of many neural and endocrine peptides. Alpha-amidation involves two sequential reactions, both of which are catalyzed by separate catalytic domains of the enzyme. The first step, catalyzed by peptidyl alpha-hydroxylating monoxygenase (PHM) domain, is the copper-, ascorbate-, and O2- dependent stereospecific hydroxylation (with S stereochemistry) at the alpha-carbon (C-alpha) of the C-terminal glycine of the

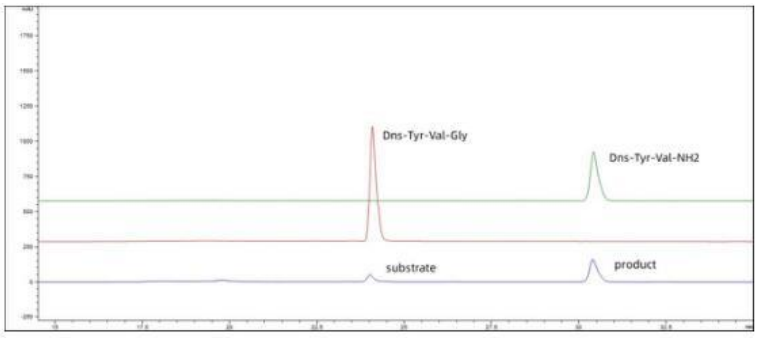
peptidylglycine substrate. The second step, catalyzed by the peptidylglycine amidoglycolate lyase (PAL) domain, is the zinc-dependent cleavage of the N-C-alpha bond, producing the alpha-amidated peptide and glyoxylate. Similarly, catalyzes the two-step conversion of an N-fatty acylglycine to a primary fatty acid amide and glyoxylate.

电泳图（SDS-PAGE image）



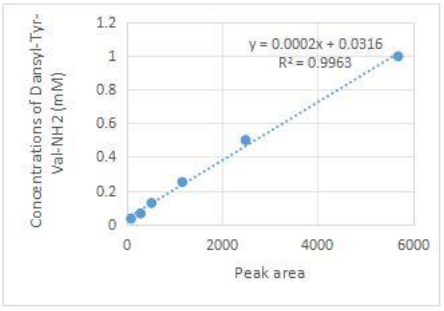
Greater than 90% as determined by reducing SDS-PAGE.

活性图（Bioactivity image）



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 2. The reaction product compared with standard Dns-Tyr-Val-Gly and Dns-Tyr-Val-NH2. Figure 3. The sandard curve of Dns-Tyr-Val-NH2



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

注意事项（Note）

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