Active Peptidylglycine Alpha Amidating Monooxygenase (PAM)

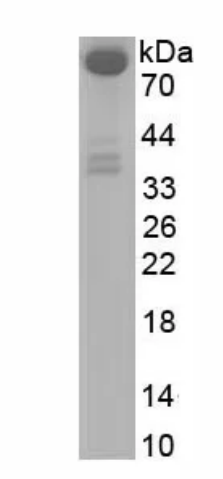
Catalog#:PHR12862

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 95% as determined by reducing SDS-PAGE.
内毒素（Endotoxin level）	Please contact with the lab for this information
蛋白构建（Construction）	【Phe36-Val820】
Accession #	P14925
表达宿主（Host）	293F
种属（Species）	Rattus norvegicus (Rat)
预测分子量（Predicted MW）	89.5 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 95% as determined by reducing SDS-PAGE.

活性图（Bioactivity image）

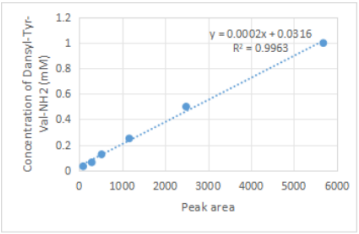


Figure 1. The standard curve of Dns-Tyr-Val-NH₂

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

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. 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.

注意事项（Note）

For Research Use Only , Not for Diagnostic Use.