Rat PAM protein, His tag (active)

Cat. No. GTX00374-pro

Applications	Functional Assay
Species	Rat

Package
10 µg

Applications

Application Note

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 preformed in 1mL containing 100 mM MES/KOH (pH 6.0), 30 mM KI, 30 mM KCl, 1µmol/L cupric sulfate, 100 µg/ml catalase, 1% (v/v) ethanol, 0.001% (v/v) Triton X-100, 10 mM ascorbate, 0.35 mM/L Dns-Tyr-Val-Gly (0.2 mg/ml) and initiated by addition various concentrations of PAM (0.1 µg/ml, 1 µg/ml, 5 µg/ml). Incubated at 37°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°C employing a Agilent ZORBAX Poroshell SB C18 column (9.4×250mm, 5µm), the flow rate was 1 ml/min. The mobile phase consisited of 100 mM sodium acetate (pH 6.5) and 35min linear gradient of 10-90% acetonitrile. The reaction product compared with standard Dns-Tyr-Val-Gly and Dns-Tyr-Val-NH2. After 30min later, the substrate have been hydrolyzed when the PAM was 5µg/ml. The retention time of Dns-Tyr-Val-Gly and Dns-Tyr-Val-NH2 is 24.315 and 30.806 respectively.

Observed MW (kDa)	88 kDa.

Properties

Form	Lyophilized powder
Buffer	Reconstitute with 10mM PBS (pH7.4) to 0.1-1.0mg/ml. Do not vortex. Lyophilized from PBS (pH7.4), 0.01% SKL, 1mM DTT, 5% Trehalose.
Preservative	ProClin 300
Storage	For short-term storage (1-2 weeks), store at 4°C. For long-term storage, store at -20°C or below. After reconstitution, keep as concentrated solution. Avoid freeze-thaw cycles.
Region/Sequence	N-terminal His-Tag; Phe36~Val820 (NP_037132.2)
Expression System	HEK293 cells
Purity	> 95%
Endotoxin	< 1 EU/µg
Conjugation	Unconjugated
Note	For laboratory use only. Not for any clinical, therapeutic, or diagnostic use in humans or animals. Not for animal or human consumption.

For full product information, images and publications, please visit our website.

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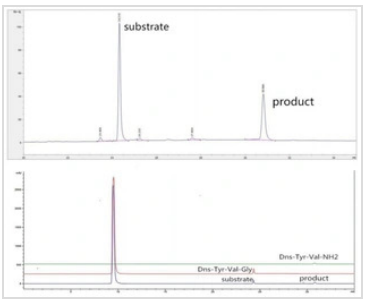
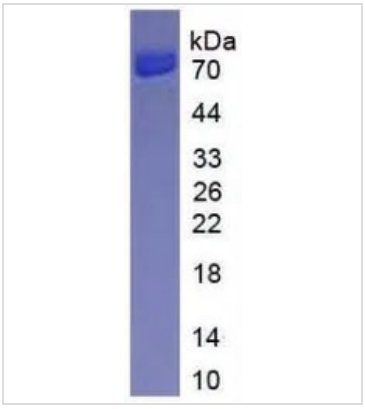
DATA IMAGES



GTX00374-pro Functional Assay Image

Hydrolysis assay of Dns-Tyr-Val-Gly by GTX00374-pro Rat PAM protein (active) for 30 min. The substrate (Dns-Tyr-Val-Gly) have been hydrolyzed to product (Dns-Tyr-Val-NH2) when the Rat PAM protein (active) was 5 µg/ml. The retention time of Substrate and Product are 24.315 and 30.806, respectively.
Upper: Activity assay of Rat PAM protein (active) by HPLC.
Lower: The reaction products compared with standard Dns-Tyr-Val-Gly and Dns-Tyr-Val-NH2.



GTX00374-pro Image

SDS-PAGE analysis of GTX00374-pro Rat PAM protein (active).

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