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Product Datasheet

Rat PAM protein, His tag (active), Unconjugated GTX00374-PRO

Article Name	Rat PAM protein, His tag (active), Unconjugated
Biozol Catalog Number	GTX00374-PRO
Supplier Catalog Number	GTX00374-pro
Alternative Catalog Number	GTX00374-PRO-10
Manufacturer	GeneTex
Category	Proteine/Peptide
Application	FA
Species Reactivity	Rat
Conjugation	Unconjugated
NCBI	25508
UniProt	P14925
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, ProClin 300.
Expression System	HEK293 cells
Form	Lyophilized powder
Sequence	N-terminal His-Tag, Phe36~Val820 (NP_037132.2)

Application Notes

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 37C 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 25C employing a Agilent ZORBAX Poroshell SB C18 column (9.4*250mm, 5μm), the flow rate was 1 ml/min. The mobile phase consisted 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.