Recombinant Human Peptidylglycine α-Amidating Monooxygenase/PAM

Catalog Number: 4837-AM

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Met1-Val817, with a C-terminal 10-His tag Accession # P19021
N-terminal Sequence Analysis	Phe21
Predicted Molecular Mass	90 kDa

SPECIFICATIONS

SDS-PAGE	79-92 kDa, reducing conditions
Activity	Measured by its ability to convert Hippurate to Benzamide and Glyoxylate. The specific activity is >1000 pmol/min/µg, as measured under the described conditions. See Activity Assay Protocol on www.RnDSystems.com
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
Formulation	Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM MES, pH 6.0
- Recombinant Human Peptidylglycine α–Amidating Monooxygenase/PAM (rhPAM) (Catalog # 4837-AM)
- Substrate: Sodium Hippurate [0.5 M Hippuric Acid (Sigma, Catalog # 112003) in 0.5 M NaOH]
- Standard: Sodium Glyoxylate (R&D Systems, please inquire)
- Glyoxylate Development Solution (Exclusive to R&D Systems, please inquire)
- Catalase (Sigma, Catalog # C30), 100,000 units/mL stock in 50 mM MES, pH 6.5
- L-Ascorbic Acid (Sigma, Catalog # 255564), 0.5 M stock in deionized water
- Copper (II) Chloride (Sigma, Catalog # 222011), 1 M stock in deionized water
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute the standard to 2000 µM in Assay Buffer. Prepare the standard curve by performing six ½ serial dilutions of the 2000 µM standard. The standard curve has a range of 0.625 to 40 nmol per well.
2. Dilute rhPAM to 5 ug/mL in cold Assay Buffer. Keep on ice.
3. Dilute Substrate to 20 mM in Assay Buffer.
4. Prepare a Reaction Mixture containing 4000 Units/mL of Catalase, 0.2 µM of Copper Chloride and 10 mM of Ascorbic Acid in cold Assay Buffer. Keep on ice. (Note: Dilute Copper Chloride to 10 µM in deionized water before adding to the mixture to prevent precipitation.)
5. In microtubes, combine 75 µL of rhPAM with 150 µL of Reaction Mixture. As a Substrate Blank, combine 75 µL of Assay Buffer with 150 µL of Reaction Mixture. For the standard curve, combine 75 µL of each dilution of the standard curve with 150 µL of Reaction Mixture.
6. Start the reaction by adding 75 µL of 20 mM Substrate to all vials.
7. Incubate at 37 °C for 1 hour.
8. Add 300 µL of Glyoxylate Development Solution to each vial, mix briefly and incubate at 95–100 °C for exactly 2 minutes.
9. Cool the vials on ice for 3 minutes.
10. Into the plate, pipette 160 µL of Substrate Blank, each dilution of the standard curve, and reactions to empty wells in triplicate.
11. Read plate in endpoint mode at excitation and emission wavelengths of 330 nm and 415 nm (top read), respectively.
12. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Glyoxylate produced* (nmol)} \times (1000\ \text{pmol/nmol})}{\text{Incubation time (min)} \times \text{amount of enzyme (µg)}}$$

*Derived from a sodium glyoxylate standard curve using linear fitting and adjusted for Substrate Blank.

Final Assay Conditions

Per Reaction:

- rhPAM: 0.1 µg
- Standard Curve: 0.625, 1.25, 2.5, 5, 10, 20, 40 nmol

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 6 months from date of receipt, -70 °C as supplied. ● 3 months, -70 °C under sterile conditions after opening.

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BACKGROUND

Pedptidylglycine α-Amidating Monooxygenase (PAM) catalyzes the C-terminal amidation that is required for the function of a number of peptide hormones (1). PAM possesses two enzymatic activities on a single polypeptide chain (2), due to the presence of a peptidylglycine α-hydroxylating monooxygenase (PHM) domain and a peptidyl-α-hydroxyglycine α-amidating lyase (PAL) domain. The C-terminal glycines of precursor peptides are hydroxylated at the glycine α carbon by the PHM activity in a reaction that requires ascorbate, then the PAL activity completes the amidation, releasing glyoxylate in the process. PAM is required for the biosynthesis of peptides such as Substance P, neuropeptide Y, oxytocin, vasopressin, and calcitonin (3). PAM is highly expressed in tissues that synthesize bioactive peptides, such as the thyroid and pituitary glands. The enzyme is generally stored in secretory granules, but soluble secreted forms have been observed (3). Recombinant human PAM was expressed as a C-terminally truncated protein lacking its transmembrane and cytosolic domains to facilitate its secretion.

References:

1. Kizer J.S. *et al.* (1986) Endocrinol. **118**:2262.
2. Perkins S.N. *et al.* (1990) Biochem. Biophys. Res. Commun. **171**:926.
3. Eipper B.A. *et al.* (1993) Protein Sci. **2**:489.

PRODUCT SPECIFIC NOTICES

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