Recombinant Human Aminopeptidase B/RNPEP

Catalog Number: 8089-ZN

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived Met1-Ser650, with a C-terminal 10-His tag Accession # Q9H4A4
N-terminal Sequence Analysis	Inconclusive: Met1 predicted. Protein identity confirmed by MS analysis of tryptic fragments.
Predicted Molecular Mass	74 kDa

SPECIFICATIONS

SDS-PAGE	60-70 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Arg-7-amido-4-methylcoumarin (Arg-AMC). The specific activity is >8,000 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.01 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, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 100 mM KCl, 1 mM DTT, pH 7.5
- Recombinant Human Aminopeptidase B/RNPEP (rhRNPEP) (Catalog # 8089-ZN)
- Substrate: H-Arg-AMC (Chem-Impex, Catalog # 05859), 10 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhRNPEP to 0.2 ng/µL in Assay Buffer.
2. Dilute Substrate to 400 µM in Assay Buffer.
3. Load 50 µL of 0.2 ng/µL rhRNPEP to a plate in triplicate, and start the reaction by adding 50 µL of 400 µM Substrate. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of Substrate.
4. Read at excitation and emission wavelengths of 380 nm and 460 nm, respectively in kinetic mode for 5 minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (RFU/min) x Conversion Factor** (pmol/RFU)}}{\text{amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard 7-amino, 4-Methyl Coumarin (Sigma, Catalog # A-9891).

Final Assay Conditions

Per Well:

- rhRNPEP: 0.01 µg
- Substrate: 200 µM

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. 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, -20 to -70 °C as supplied. ● 3 months, -20 to -70 °C under sterile conditions after opening.

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BACKGROUND

Aminopeptidase B (APB; also known as aminopeptidase basic, Arginine aminopeptidase and RNPEP) is a monomeric, secreted member of the metallopeptidase M1 family of enzymes (1-2). Members of the M1 family are often associated with mitosis, and are characterized by the presence of a catalytic domain that consists of a GxMxN exopeptidase motif, coupled to an extended Zn-binding HExxH[18x]E sequence (1, 3). Although Zn-dependent, it is activated by divalent cations such as Ni, Mn and Co. APB is widely expressed, perhaps even ubiquitously, and has been reported in a number of distinct cell types. These include hepatocytes (4), skeletal muscle cells (5), macrophages and neutrophils (6), pancreatic islet α-cells (7), anterior lobe pituitary basophils (8), and adrenal medullary chromaffin cells (8). APB has been identified in multiple subcellular locations, including an extracellular association with the plasma membrane, within secretory granules of neuroendocrine cells, and through an NLS, within the cell nucleus (1, 7-11). APB appears to act in concert with at least one other peptidase during the processing of propeptides into active or mature peptides. The activity attributed to APB involves the preferential cleavage of the basic amino acids (aa) Arg and Lys from the N-terminus of partially processed proforms (9). This activity is reported to occur in α-cell granules where an NRD convertase:APB cooperation converts glucagon into miniglucagon (aa 19-29), and in adrenal medullary chromaffin cell granules where a cathepsin L:APB cooperation converts proenkephalin into Met-enkephalin (7, 8). The human APB precursor is 650 aa in length. It contains an atypical 28 aa signal sequence plus a 622 aa mature region that contains a peptidase region over aa 24-634 (10, 12). A leukotriene A4 hydrolase has also been described between aa 500-644. Mature APB is reported to run as a 72-76 kDa protein on SDS-PAGE (5, 8, 10, 11). There are potential isoform variants. One shows a 12 aa substitution for aa 11-14, while another may utilize an alternate start site at Met291. Mouse and rat APB share 89% aa sequence identity with human APB.

References:

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