Recombinant Human Aminopeptidase N/CD13

Catalog Number: 3815-ZN

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Lys69-Lys967, with a C-terminal 10-His tag Accession # NP_001141
N-terminal Sequence Analysis	Lys69
Predicted Molecular Mass	104 kDa

SPECIFICATIONS

SDS-PAGE	131 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Ala-7-amido-4-methylcoumarin (Ala-AMC). The specific activity is >2,500 pmol/min/µg, as measured under the described conditions.
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 silver stain.
Formulation	Supplied as a 0.2 µm filtered solution in MES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, pH 7.0
- Recombinant Human Aminopeptidase N/CD13 (rhCD13) (Catalog # 3815-ZN)
- Substrate: Ala-AMC (Bachem, Catalog # I-1410)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: Spectramax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhCD13 to 0.2 µg/mL in Assay Buffer.
2. Dilute Substrate to 200 µM in Assay Buffer.
3. Load 50 µL of 0.2 µg/mL rhCD13 into a plate, and start the reaction by adding 50 µL of 200 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 (top read), 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 # A9891).

Final Assay Conditions

Per Well:

- rhCD13: 0.010 µg
- Substrate: 100 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.

BACKGROUND

The human ANPEP gene encodes aminopeptidase N (APN), which is also known as microsomal aminopeptiase, alanyl aminopeptidase, aminopeptidase M, CD13, or membrane protein p161 (1-3). The deduced amino acid sequence of human APN consists of a short cytoplasmic tail (residues 2 to 8), a transmembrane region (residue 9 to 32), a Ser/Thr rich region and a zinc metalloprotease domain (residues 69 to 966). Widely expressed in many cells, tissues and species, APN cleaves the N-terminal amino acids from bioactive peptides, leading to their inactivation or degradation. The roles of APN in many fields, such as neuroscience, hematopoeitic cells, immune system, angiogenesis, cancer and viral infection, have been reviewed (3).

References:

1. Olsen, J. *et al.* (1988) FEBS Lett. **238**:307.
2. Look, A.T. *et al.* (1989) J. Clin. Invest. **83**:1299.
3. Turner, A.J. (2004) in *Handbook of Proteolytic Enzymes* (ed. Barrett, *et al.*) pp. 289, Academic Press, San Diego.

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