Recombinant Human ENPP-2/Autotaxin
Catalog Number: 5255-EN

DESCRIPTION
Source Mouse myeloma cell line, NS0-derived human ENPP-2/Autotaxin protein
Asp49-Ile863, with an N-terminal 6-His tag
Accession # Q13822

N-terminal Sequence Analysis
His

Predicted Molecular Mass 94 kDa

SPECIFICATIONS
SDS-PAGE 99 kDa and 103 kDa, migrates as a doublet under reducing conditions

Activity Measured by its ability to cleave Bis (p-Nitrophenyl) Phosphate (BPNPP).
The specific activity is >8,000 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 Tris and NaCl.
See Certificate of Analysis for details.

Activity Assay Protocol

Materials
- Assay Buffer: 50 mM Tris, 10 mM CaCl₂, 5 mM MgCl₂, 0.02% Brij-35 (w/v), pH 8.5
- Recombinant Human ENPP-2/Autotaxin (rhENPP-2) (Catalog # 5255-EN)
- Substrate: Bis(p-Nitrophenyl) Phosphate Sodium Salt (BPNPP) (Sigma, Catalog # N3002), 40 mM stock in deionized water (Note: Heating may be necessary to solubilize Substrate.)
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay
1. Dilute rhENPP-2 to 0.1 ng/µL in Assay Buffer.
2. Dilute Substrate BPNPP to 2 mM in Assay Buffer.
3. Load into the wells of a microplate 50 µL of 0.1 ng/µL rhENPP-2 and start the reaction by adding 50 µL of 2 mM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL Substrate.
4. Read at 400 nm (absorbance) in kinetic mode for 5 minutes.
5. Calculate specific activity:

   Specific Activity (pmol/min/µg) = \( \frac{Adjusted \ V_{max}^* \ (OD/min) \times Conversion \ Factor** \ (pmol/OD)}{amount \ of \ enzyme \ (µg)} \)

   *Adjusted for Substrate Blank

   **Derived using calibration standard p-Nitrophenol (Sigma-Aldrich, Catalog # 241326).

Final Assay Conditions Per Well:
- rhENPP-2: 0.005 µg
- Substrate: 1 mM

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
ENPP-2, also known as Autotaxin, belongs to the ectonucleotide pyrophosphatase/phosphodiesterase (NPP) family. Some NPPs hydrolyze phosphates from nucleotides and their derivatives. ENPP-2 shares 40 - 50% identity to ENPP1 & 3, all of which contain a N-terminal intracellular domain, a single transmembrane domain and a large extracellular domain that includes a catalytic domain, two somatomedin-B-like domains, and a C-terminal nuclease-like domain (1). Unlike ENPP-1 and ENPP-3, ENPP-2 has weak activity against nucleotides, but exhibits a lysophospholipase D activity which allows the formation of lysophosphatidic acid (LPA) and choline from lysophosphatidylcholine (2). The hydrolysis of nucleotides and lysophospholipids by ENPP-2 is mediated by a single catalytic site (2). Evidence shows LPA and sphingosine 1-phosphate to be specific inhibitors of ENPP-2 (3). ENPP-2 was originally found to stimulate tumor cell motility and has since been found to enhance tumor invasion and metastasis (4) and to be up-regulated in several types of carcinomas including breast and lung (5).

References:

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PRODUCT SPECIFIC NOTICES

This product may be covered by one or more of the following US issued patents 5,449,753; 5,731,167; 6,084,069; 6,417,338 and US pending applications.