Recombinant Human ENPP-6

Catalog Number: 8489-EN

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Arg23-Ser419, with a C-terminal 10-His tag Accession # Q6UWR7
N-terminal Sequence Analysis	Arg23
Predicted Molecular Mass	47 kDa

SPECIFICATIONS

SDS-PAGE	49-60 kDa, reducing conditions
Activity	Measured by its ability to cleave O-(4-Nitrophenylphosphoryl) choline. The specific activity is >4,000 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 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 Tris, 0.5 M NaCl, pH 9.0
- Recombinant human ENPP-6 (rhENPP-6) (Catalog # 8489-EN)
- Substrate: O-(4-Nitrophenylphosphoryl) choline (Sigma, Catalog # N5879), 500 mM stock in deionized water
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhENPP-6 to 0.4 µg/mL in Assay buffer.
2. Dilute room temperature Substrate to 2 mM in Assay buffer.
3. Load 50 µL of 0.4 ng/µL rhENPP-6 in a clear strip well plate.
4. Start the reaction by adding 50 µL of 2 mM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL Substrate.
5. Read at 405 nm (absorbance) in kinetic mode for 5 minutes.
6. Calculate specific activity:

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

*Adjusted for Substrate Blank.

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

Final Assay Conditions

Per Well:

- rhENPP-6: 0.02 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.

Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

Recombinant Human ENPP-6

Catalog Number: 8489-EN

BACKGROUND

Ectonucleotide Pyrophosphatase/Phosphodiesterase 6 (ENPP-6) is a choline-specific glycerophosphodiester phosphodiesterase. It is a member of a family of membrane-linked glycoproteins that, at an alkaline pH, hydrolyze pyrophosphate or phosphodiester bonds in a variety of extracellular compounds including nucleotides, lysophospholipids, and choline phosphate esters (1). ENPP-6 is a GPI-linked protein that is synthesized as a 440 amino acid (aa) precursor and has a predicted molecular weight of approximately 55 kDa (2). Its extracellular region contains a catalytic domain that is nearly 400 aa in length and shares 88% aa sequence identity with the mouse and rat orthologs (1, 3). A soluble form of ENPP-6 can be proteolytically shed and associate into a disulfide-linked homodimer (2, 4).

The catalytic domain of ENPP-6 specifically recognizes the phosphocholine part of its substrate (2, 3). ENPP-6 has been shown to display preference for choline-containing phospholipids or phosphodiesters such as lysophosphatidylcholine (LPC), glycerophosphorylcholine (GPC), sphingosylphosphorylcholine (SPC), Platelet-Activating Factor (PAF), and lysoPAF (3). Furthermore, ENPP-6 shows preference for LPC with short (12:0 and 14:0) or polyunsaturated (18:2 and 20:4) fatty acids (3). *In vitro*, ENNP-6 has been shown to efficiently hydrolyze the classical substrate for phospholipase C, p-nitrophenyl phosphorylcholine, but not the classical nucleotide phosphodiesterase substrate, p-nitrophenyl thymidine 5'-monophosphate (3). ENPP-6 is predominantly expressed in brain, where it is localized to myelin and kidney, with lesser expression being found in the heart (3, 5). ENPP-6 expression in the brain has been shown to be regulated by Thyroid Hormone and iron (6, 7). Additionally, in rat brain, ENPP-6 expression has been shown to be up-regulated during oligodendrocyte differentiation (8, 9).

References:

1. Stefan, C. *et al.* (2005) Trends Biochem. Sci. **30**:542.
2. Greiner-Tollersrud, O.K. (2014) Neurochem. Res. **39**:2025.
3. Sakagami, H. *et al.* (2005) J. Biol. Chem. **280**:23084.
4. Greiner-Tollersrud, L. *et al.* (2013) Neurochem. Res. **38**:300.
5. Jahn, O. *et al.* (2009) Mol. Neurobiol. **40**:55.
6. Royland, J.E. *et al.* (2008) J. Neuroendocrinol. **20**:1319.
7. Bastian, T.W. *et al.* (2012) Endocrinology **153**:5668.
8. Dugas, J.C. *et al.* (2006) J. Neurosci. **26**:10967.
9. Cahoy, J.D. *et al.* (2008) J. Neurosci. **28**:264.

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 **Canada** TEL 855 668 8722 **China** TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449