Recombinant Mouse ENPP-4

Catalog Number: 8996-EN

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived Tyr19-Ala410, with a C-terminal 6-His tag Accession # Q8BTJ4
N-terminal Sequence Analysis	Tyr19
Predicted Molecular Mass	45 kDa

SPECIFICATIONS

SDS-PAGE	56-74 kDa, reducing conditions
Activity	Measured by its ability to hydrolyze thymidine 5'-monophosphate p-nitrophenyl ester. The specific activity is >22,000 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 visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
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, pH 7.5
- Recombinant Mouse ENPP-4 (rmENPP-4) (Catalog # 8996-EN)
- Substrate: Thymidine 5'-monophosphate p-nitrophenyl ester (Sigma, Catalog # T4510), 100 mM stock in deionized water
- NaOH, 0.2 M in deionized water
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rmENPP-4 to 0.1 ng/µL in Assay Buffer.
2. Dilute Substrate to 10 mM in Assay Buffer.
3. Load 50 µL of 0.1 ng/µL rmENPP-4 in a clear strip well plate, and start the reaction by adding 50 µL of 10 mM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL 10 mM Substrate.
4. Incubate sealed plate at room temperature for 30 minutes.
5. Stop reactions by adding 100 µL of 0.2 M NaOH to all wells, including Substrate Blank wells.
6. Read at 410 nm (absorbance) in endpoint mode.
7. Calculate specific activity:

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

*Adjusted for Substrate Blank

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

Final Assay Conditions

Per Reaction:

- rmENPP-4: 0.005 µg
- Substrate: 5 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

Ectonucleotide pyrophosphatase/phosphodiesterase 4 (ENPP-4 or NPP4) belongs to a group of ecto-enzymes which regulate the availability of extracellular nucleotides (1). This enzyme family forms a subgroup of a larger family that also includes arylsulfatases, phosphopentomutases, 2,3-bisphosphoglycerate-independent phosphoglycerate mutases (iPGM), and alkaline phosphatases (2). Mature mouse ENPP-4 consists of a 392 amino acid (aa) ectodomain that contains the catalytic domain with a zinc-coordinated substrate binding pocket, a 21 aa transmembrane segment, and a 25 aa cytoplasmic tail (3). It shares 86% and 90% aa sequence identity with human and rat ENPP-4, respectively. Alternative splicing generates a short isoform with a 32 aa deletion in the phosphodiesterase domain. ENPP-4 hydrolyzes phosphodiester bonds in nucleotides with a preference for adenine nucleotides (3). It cleaves the diadenosine compounds Ap3A and Ap4a which are released from the dense granules of thrombin-activated platelets (3, 4). These reactions generate AMP and ADP from Ap3A cleavage, and AMP and ATP from Ap4A cleavage (4). ENPP-4 is expressed on the surface of vascular endothelial cells where its activity prolongs platelet aggretation and contributes to thrombus formation (4).

References:

1. Zimmermann, H. *et al.* (2012) Purinergic Signal. **8**:437.
2. Gijsbers, R. *et al.* (2001) J. Biol. Chem. **276**:1361.
3. Albright, R.A. *et al.* (2014) J. Biol. Chem. **289**:3294.
4. Albright, R.A. *et al.* (2012) Blood **120**:4432.

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