Recombinant Human CD39L3/ENTPD3

Catalog Number: 4400-EN

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Gln44-Pro485, with a C-terminal 6-His tag Accession # O75355
N-terminal Sequence Analysis	No results obtained: Gln44 predicted
Predicted Molecular Mass	50 kDa

SPECIFICATIONS

SDS-PAGE	65-80 kDa, reducing conditions
Activity	Measured by its ability to hydrolyze the 5'-phosphate groups from the substrate adenosine-5'-triphosphate (ATP). The orthophosphate product is measured by a Malachite Green Phosphate Detection Kit (Catalog # DY996). The specific activity is >70,000 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, $CaCl_2$, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM Tris, 5 mM $CaCl_2$, pH 7.5
- Recombinant Human CD39L3/ENTPD3 (rhCD39L3) (Catalog # 4400-EN)
- Substrate: ATP (Sigma, Catalog # A-7699), 10 mM in deionized water
- Malachite Green Phosphate Detection Kit (Catalog # DY996)
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhCD39L3 to 0.02 µg/mL in Assay Buffer.
2. Prepare a standard curve from 1 M Phosphate Standard by adding 10 µL of the 1 M Phosphate Standard to 990 µL of Assay Buffer for a 10 mM stock. Continue by adding 10 µL of the 10 mM Phosphate stock to 990 µL of Assay Buffer for a 100 µM stock. (This is the first dilution to use as a standard).
3. Perform six additional one-half serial dilutions of the 100 µM Phosphate stock in Assay Buffer. The standard curve has a range of 0.039 to 2.5 nmol per well.
4. Load 25 µL of 0.02 µg/mL rhCD39L3 and the standard curve into a plate. Include a Substrate Blank containing Assay Buffer.
5. Dilute the Substrate to 100 µM in Assay Buffer.
6. Add 25 µL of the 100 µM Substrate to all wells and mix well.
7. Cover the plate with parafilm or a plate sealer and incubate at 37 °C for 30 minutes.
8. Add 10 µL of the Malachite Green Reagent A to all wells. Mix and incubate for 10 minutes at room temperature.
9. Add 10 µL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
10. Read plate at 620 nm (absorbance) in endpoint mode.
11. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Phosphate released* (nmol) x (1000 pmol/nmol)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Derived from the phosphate standard curve using linear or 4-parameter fitting and adjusted for Substrate Blank.

Final Assay Conditions

Per Well:

- rhCD39L3: 0.0005 µg
- Substrate: 35.7 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

Ectonucleoside triphosphate diphosphohydrolase-3 (NTPDase-3), encoded by the ENTPD3 gene and also known as CD39L3, is an integral membrane protein with an extracellular active site (1). Recombinant human NTPDase-3 was expressed as a protein lacking its N- and C-terminal transmembrane domains, resulting in the secretion of the soluble ectodomain. NTPDase-3 hydrolyzes the β- and γ-phosphate residues of nucleotides, preferring ATP, ADP, UTP, and UDP as substrates (1). Through its hydrolysis of extracellular nucleotides, NTPDase-3 plays a role in the regulation of purinergic signaling (2). The enzyme is expressed at its highest levels in brain, pancreas, spleen and prostate tissues (3).

References:

1. Lavoie, E.G. *et al.* (2004) Biochem. Pharmacol. **67**:1917.
2. Crawford, P.A. *et al.* (2007) Arch. Biochem. Biophys. **457**:7.
3. Chadwick, B.P. and A.M. Frischauf (1998) Genomics **50**:357.

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