Recombinant Mouse CD39/ENTPD1

Catalog Number: 4398-EN

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Thr38-Ile478, with a C-terminal 6-His tag Accession # Q921Q6
N-terminal Sequence Analysis	Thr38
Predicted Molecular Mass	50 kDa

SPECIFICATIONS

SDS-PAGE	60-70 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 >25,000 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, NaCl, $CaCl_2$ 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 Mouse CD39/ENTPD1 (rmCD39) (Catalog # 4398-EN)
- Substrate: Adenosine triphosphate (ATP) (Sigma, Catalog # A-7699), 10 mM stock 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. Prepare a standard curve from the 1 M Phosphate Standard supplied in the malachite green phosphate detection kit. First, add 10 µL of the 1 M Phosphate Standard to 990 µL of Assay Buffer for a 10 mM stock. Then, add 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). Finally, perform six additional two-fold serial dilutions of the 100 µM phosphate stock. The standard curve has a range of 0.039 to 2.5 nmol per well.
2. Dilute rmCD39 to 0.02 µg/mL in Assay Buffer.
3. Transfer to a plate (in duplicate) 25 µL of standard curve, diluted rmCD39 at 0.02 µg/mL, and blanks (Assay Buffer).
4. Dilute Substrate to 100 µM in Assay Buffer.
5. Add 25 µL of the Substrate to all wells. Mix well.
6. Cover the plate with parafilm or a plate sealer and incubate at 37 °C for 30 minutes.
7. After incubation, add 10 µL of the Malachite Green Reagent A to each sample, standard, and blank. Mix and incubate for 10 minutes at room temperature.
8. Add 10 µL of the Malachite Green Reagent B to each sample, standard, and blank. Mix and incubate for 20 minutes at room temperature.
9. Read plate at 620 nm (absorbance) in endpoint mode.
10. 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 Reaction:

- rmCD39: 0.0005 µg
- Substrate: 50 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.

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BACKGROUND

Ectonucleoside triphosphate diphosphohydrolase-1 (NTPDase-1) is an integral membrane protein with an extracellular active site. Recombinant mouse NTPDase-1 was expressed as a protein lacking its N- and C-terminal transmembrane domains, resulting in the secretion of the soluble ectodomain. NTPDase-1 was originally described as CD39, a B lymphocyte cell surface marker (1), but it is also present on the surface of natural killer cells, T cells, and some endothelial cells (2). NTPDase-1 hydrolyzes the β- and γ phosphate residues of nucleotides, preferring ATP as the substrate. Through its hydrolysis of extracellular nucleotides, NTPDase-1 plays a role in the regulation of purinergic signaling (3). NTPDase-1 is involved in the processes of thromboregulation and vascular inflammation (4). The administration of soluble NTPDase-1 may have therapeutic applications for the treatment of some vascular and transplantation-associated diseases (5).

References:

1. Rowe, M. *et al.* (1982) Int. J. Cancer **29**:373.
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4. Marcus, A.J. *et al.* (2005) Semin. Thromb. Hemost. **31**:234.
5. Robson, S.C. *et al.* (2005) Semin. Thromb. Hemost. **31**:217.

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