Recombinant Cynomolgus Monkey CD39/ENTPD1 His-tag

Catalog Number: 11092-EN

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived cynomolgus monkey CD39/ENTPD1 protein Thr45-Val485, with a C-terminal 6-His tag Accession # EHH64904.1
N-terminal Sequence Analysis	Thr45
Predicted Molecular Mass	51 kDa

SPECIFICATIONS

SDS-PAGE	60-77 kDa, under 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 >15,000 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 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 Cynomolgus Monkey CD39 (rcynoCD39) (Catalog # 11092-EN)
- Adenosine triphosphate (ATP) (Sigma, Catalog # A7699), 10 mM stock in deionized water
- Malachite Green Phosphate Detection Kit (Catalog # DY996)
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Prepare a standard curve from the 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.
2. 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. Prepare standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock in Assay Buffer. The standard curve has a range of 0.078 to 5 nmol per well.
4. Dilute rcynoCD39 to 0.04 µg/mL in Assay Buffer.
5. Dilute ATP to 400 µM in Assay Buffer.
6. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
7. Load 25 µL of the 0.04 µg/mL rcynoCD39 into empty wells of the plate. Include a Control containing 25 µL of Assay Buffer.
8. Start the reactions by adding 25 µL of 400 µM ATP to all the wells, excluding the standard curve and curve blank.
9. Incubate sealed plate at 37 °C for 20 minutes.
10. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
11. Add 100 µL of deionized water to all wells. Mix briefly.
12. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
13. Read plate at 620 nm (absorbance) in endpoint mode.
14. 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 Control.

Final Assay Conditions

Per Reaction:

- rcynoCD39: 0.001 µg
- ATP: 200 µM

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. 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.

DATA

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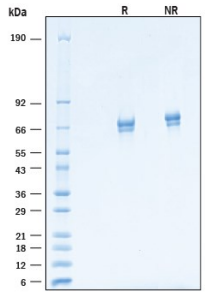
Recombinant Cynomolgus Monkey CD39/ENTPD1 His-tag

Catalog Number: 11092-EN

SDS-PAGE



Recombinant Cynomolgus Monkey CD39/ENTPD1 His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant Cynomolgus Monkey CD39/ENTPD1 His-tag Protein (Catalog # 11092-EN) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 60-77 kDa.

BACKGROUND

CD39, also known as ectonucleoside triphosphate diphosphohydrolase-1 (NTPDase-1), is the prototypical member of the NTPDase family. CD39 is a metal-dependent, homodimeric enzyme bound to the cell membrane through two transmembrane domains present at each termini (1-3). The extracellular loop contains two major domains with an active site and apyrase conserved regions (ACRs) and changes conformation dynamically during catalysis (1-4). Although first described as a B lymphocyte cell surface marker (5), CD39 is also present on the surface of natural killer cells, T cells, and some endothelial cells (6). CD39 hydrolyzes the beta - and gamma phosphate residues of nucleotides to terminate purinergic signaling and is uniquely capable of processive hydrolysis of extracellular ATP to AMP without release of the ADP intermediate (3, 7, 8) as the initial rate-limiting steps in the production of extracellular adenosine (9). CD39 functional activity leads to interest in CD39 as a pharmaceutical therapeutic target for several disease models including inflammation, cancer, and immunosuppression (3,4,9,10). Recombinant cynomolgus NTPDase-1 was expressed as a protein lacking its N- and C-terminal transmembrane domains, resulting in the secretion of the soluble recombinant NTPDase-1 ectodomain.

References:

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