Recombinant Human CD39L2/ENTPD6

Catalog Number: 4399-EN

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Lys61-Ser484, with an N-terminal 6-His tag Accession # O75354
N-terminal Sequence Analysis	His
Predicted Molecular Mass	47 kDa

SPECIFICATIONS

SDS-PAGE	50-55 kDa, reducing conditions
Activity	Measured by its ability to hydrolyze the 5'-phosphate groups from the substrate guanosine-5'-diphosphate (GDP). The orthophosphate product is measured by a Malachite Green Phosphate Detection Kit (Catalog # DY996). The specific activity is >30,000 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, 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 Human CD39L2/ENTPD6 (rhCD39L2) (Catalog # 4399-EN)
- Substrate: GDP (Sigma, Catalog # G-7127), 100mM stock in deionized H_2O
- 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 rhCD39L2 to 0.02 µg/mL in Assay Buffer.
2. Prepare a standard curve from 1 M Phosphate Standard supplied in the Malachite Green Phosphate Detection Kit:
   a. Add 10 µL of the 1 M Phosphate Standard to 990 µL of Assay Buffer for a 10 mM stock.
   b. 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.)
   c. 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.
3. Load 25 µL of 0.02 µg/mL rhCD39L2 and the standard curve into a plate. Include a Substrate Blank containing Assay Buffer.
4. Dilute the Substrate to 100 µM in Assay Buffer.
5. Add 25 µL of the 100 µM Substrate to all wells and mix well.
6. Cover the plate with parafilm or a plate sealer and incubate at 37 °C for 20 minutes.
7. Add 10 µL of the Malachite Green Reagent A to all wells. Mix and incubate for 10 minutes at room temperature.
8. Add 10 µL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
9. Read plate at 620 nm (absorbance) in endpoint mode.
10. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Phosphate released* (nmol) x (1000 pmol/1 nmol)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

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

Final Assay Conditions

Per Well:

- rhCD39L2: 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-6 (NTPDase-6) is a secreted nucleoside phosphohydrolase of the CD39 family of enzymes (1). hNTPDase-6 displays a preference for the nucleoside-5'-diphosphates GDP and IDP over CDP and UDP (2). Nucleoside-5'-triphosphates are also hydrolyzed, but at much lower rates. hNTPDase-6 has a broad tissue distribution, its mRNA has been detected in all human tissues tested (1).

References:

1. Chadwick, B.P. and A.M. Frischauf (1998) Genomics. **50**:357.
2. Hicks-Berger, C.A. *et al.* (2000) J. Biol. Chem. **275**:34041.

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