Recombinant Mouse CD39L1/ENTPD2

Catalog Number: 5797-EN

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Cys26-Ser462, with a C-terminal 6-His tag Accession # O55026
N-terminal Sequence Analysis	Cys26
Predicted Molecular Mass	49 kDa

SPECIFICATIONS

SDS-PAGE	60-80 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 >9,000 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 Mouse CD39L1/ENTPD2 (rmCD39L1) (Catalog # 5797-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 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).
2. 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. Dilute rmCD39L1 to 0.08 µg/mL in Assay Buffer.
4. Load 25 µL of 0.08 µg/mL rmCD39L1 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 20 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:

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

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

Final Assay Conditions

Per Well:

- rmCD39L1: 0.002 µg
- Substrate: 35.7 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, -70 °C as supplied. • 3 months, -70 °C under sterile conditions after opening.

BACKGROUND

CD39L1, also known as ENTPD2 and NTPDase2, is an ecto-nucleotidase belonging to the CD39 family. It is found on the surface of vascular adventitial cells and accessory vascular cells (1). CD39L1 is a Ca^{2+}- and Mg^{2+}-dependent enzyme that activates platelets by preferentially converting ATP to ADP (2). CD39L1 plays a role in regulating thrombosis and inflammation (3). It is considered to be a therapeutic target for thromboregulation and the treatment of vascular inflammation (2, 4).

References:

1. Zimmermann, H. *et al.* 2000 Proceedings of the Second International Workshop on Ecto-ATPases and Related Ectonucleotidases:18.
2. Robson, S.C. *et al.* 2001 Drug Dev. Res. **53**:193.
3. Marcus, A.J. *et al.* 2005 Semin. Thromb. Hemost. **31**:234.
4. Sevigny, J. *et al.* 2002 Blood **99**:2801.

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Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449