Recombinant Cynomolgus Monkey 5'-Nucleotidase/CD73 His-tag

Catalog Number: 10173-EN

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived cynomolgus monkey 5'-Nucleotidase/CD73 protein Trp27-Lys547 Accession # XP_005552488.1 with a C-terminal 6-His tag
N-terminal Sequence Analysis	Trp27
Structure / Form	Dimer
Predicted Molecular Mass	59 kDa

SPECIFICATIONS

SDS-PAGE	57-75 kDa
Activity	Measured by its ability to hydrolyze the 5'-phosphate group from the substrate adenosine-5'-monophosphate (AMP). The orthophosphate product is measured by a Malachite Green Phosphate Detection Kit (Catalog # DY996). The specific activity is >6500 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	>90%, 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 $MgCl_2$, pH 7.5
- Recombinant Cynomolgus Monkey 5'-Nucleotidase/CD73 (rcynoCD73) (Catalog # 10173-EN)
- Adenosine monophosphate (AMP) (Sigma, Catalog # A1752), 5 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. 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 using Assay Buffer. The standard curve has a range of 0.078 to 5 nmoles per well.
3. Dilute Substrate to 100 µM in Assay Buffer.
4. Dilute rcynoCD73 to 0.08 µg/mL in Assay Buffer.
5. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
6. Load 25 µL of 0.08 µg/mL rcynoCD73 into empty wells of the same plate as the curve. Include a Control containing 25 µL of Assay Buffer.
7. Add 25 µL of diluted Substrate to all wells, excluding the standard curve.
8. Seal plate and incubate at 37 °C for 20 minutes.
9. Add 30 µL of the Malachite Green Reagent A to all wells used, including standard curve. Mix briefly.
10. Add 100 µL of deionized water to all wells used, including standard curve. Mix briefly.
11. Add 30 µL of the Malachite Green Reagent B to all wells used, including standard curve. Mix briefly.
12. Seal plate and incubate at room temperature for 20 minutes.
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:

- rcynoCD73: 0.002 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

CD73, known as ecto-5'-Nucleotidase, converts extracellular nucleoside 5' monophosphates to nucleosides, with AMP as its preferred substrate (1). CD73 is a zinc-dependent, 70 kDa homodimeric enzyme bound to the cell membrane through a glycosyl phophatidylinositol (GPI) anchor. It is composed of an N-terminal domain containing metal binding sites linked via small hinge region to a C-terminal domain containing the substrate binding site and dimerization interface (2). It is expressed by most cell types (3) and is widely expressed in tumor cell lines as well as upregulated in cancerous tissues (4,5). CD73 is a key enzyme responsible for a rate-limiting step in the generation of extracellular adenosine. Adenosine is a molecule that signals through activation of purinergic receptors and results in an immunosuppressive role in the tumor microenvironment (4,6). CD73 has been implicated in many pathological processes including immunomodulation and inflammation (7,8) tumor growth and metastasis (9-13) making CD73 a potential drug target in cancer. Targeting CD73 inhibition has resulted in numerous reports of favorable antitumor effects (4,5,12). Consequently, therapeutic approaches have been tested using knockdown, gene silencing and anti-CD73 therapies (11,14) as well as small molecule inhibitors (14,15).

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