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Recombinant Human 5'-Nucleotidase/CD73

Catalog Number: 5795-EN

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Trp27-Lys547, with a C-terminal 6-His tag, Trp27-Lys547 Accession # AAH65937
N-terminal Sequence Analysis	Trp27
Structure / Form	Dimer
Predicted Molecular Mass	59 kDa

SPECIFICATIONS

SDS-PAGE	61-62 kDa, reducing conditions
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 >15,000 pmol/min/µg, as measured under the described conditions. See Activity Assay Protocol on www.RnDSystems.com.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>90%, 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 $MgCl_2$, pH 7.5
- Recombinant Human 5'-Nucleotidase/CD73 (rhCD73) (Catalog # 5795-EN)
- Substrate: Adenosine monophosphate (AMP) (Sigma, Catalog # A1752), 5 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. Dilute rhCD73 to 0.04 µg/mL in Assay Buffer.
2. 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).
3. Perform six additional one-half serial dilutions of the 100 µM phosphate stock. The standard curve has a range of 0.039 to 2.5 nmol per well.
4. Load 25 µL of 0.04 µg/mL rhCD73, standard curve, and blanks (Assay Buffer) into a plate.
   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 ºC 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:

- rhCD73: 0.001 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.

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BACKGROUND

CD73, an ecto-5'-Nucleotidase, is an ectoenzyme expressed by most cell types (1). The 5'-Nucleotidase activity of CD73 converts extracellular nucleoside-5'-monophosphates to nucleosides, with AMP as the preferred substrate. CD73 is one of several enzymes responsible for the production of extracellular adenosine, a signaling molecule that is involved in responses to inflammation and tissue injury (2). CD73 is a lymphocyte maturation marker that has functions independent of its catalytic activity. These functions include the adhesion of B-cells to follicular dendritic cells and T-cell signaling (3, 4). CD73 is also a regulator of leukocyte extravasation, a function that requires its 5'-Nucleotidase activity (5). The native enzyme is a homodimer bound to the cell membrane through a glycosyl phophatidylinositol (GPI) anchor.

References:

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4. Resta, R. and Thompson. L.F. (1997) Cell Signal. **9**:131.
5. Jalkanen, S. and Salmi, M. (2008) Arterioscler. Thromb. Vasc. Biol. **28**:18.

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