**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.

**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, CaCl₂ and Glycerol. See Certificate of Analysis for details.

**Activity Assay Protocol**

**Materials**

- **Assay Buffer**: 25 mM Tris, 5 mM MgCl₂, 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.
5. Dilute Substrate to 100 μM in Assay Buffer.
6. Load 25 μL of the Substrate to all wells. 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.

**Calculate specific activity:**

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\text{Specific Activity (pmol/min/μg)} = \frac{\text{Phosphate released} (\text{nmol}) \times (1000 \text{ pmol/1 nmol})}{\text{Incubation time (min)} \times \text{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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**Recombinant Human**  
5'-Nucleotidase/CD73  
Catalog Number: 5795-EN

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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: