Recombinant Human PRPS1 His-tag

Catalog Number: 11075-PS

DESCRIPTION

Source	*E. coli*-derived human PRPS1 protein Pro2-Leu318 with a C-terminal 6-His tag Accession # P60891.2
N-terminal Sequence Analysis	Pro2
Predicted Molecular Mass	36 kDa

SPECIFICATIONS

SDS-PAGE	36 kDa
Activity	Measured by its ability to convert D-ribose 5-phosphate to 5-phospho-alpha-D-ribose. The specific activity is >25 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	>95%, 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, Glycerol and TCEP. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 10 mM $MgCl_2$, pH 7.5
- Recombinant Human Phosphoribosyl Pyrophosphate Synthetase 1 (rhPRPS-1) (Catalog # 11075-PS)
- D-Ribose 5-phosphate disodium salt hydrate (R5P) (Sigma, Catalog # R7750), 150 mM stock in deionized water
- Adenosine triphosphate (ATP) (Sigma, Catalog # A7699), 10 mM stock in deionized water
- Recombinant Human 5'-Nucleotidase/CD73 (rhCD73) (Catalog # 5795-EN)
- 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 nmol per well.
3. Prepare Reaction Mixture containing 400 µM ATP, 1 mM R5P and 0.6 µg/mL rhCD73 in Assay Buffer.
4. Dilute rhPRPS-1 to 80 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 80 µg/mL rhPRPS-1 into empty wells of the same plate as the curve. Include a Control containing 25 µL of Assay Buffer.
7. Start the reactions by adding 25 µL of Reaction Mixture to all wells, excluding the standard curve and curve blank.
8. Seal plate and incubate at room temperature 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:

- rhPRPS-1: 2 µg
- rhCD73: 0.015 µg
- ATP: 200 µM
- R5P: 500 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.

DATA

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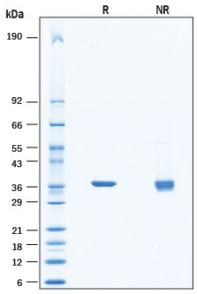
Recombinant Human PRPS1 His-tag

Catalog Number: 11075-PS

SDS-PAGE



Recombinant Human PRPS1 His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant Human PRPS1 His-tag Protein (Catalog # 11075-PS) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 36 kDa.

BACKGROUND

Ribose-phosphate pyrophosphokinase 1 (PRPS1), also known as phosphoribosyl pyrophosphate synthase I, is a highly conserved, ubiquitously expressed enzyme from the ribose-phosphate pyrophosphokinase family that catalyzes the synthesis of phosphoribosylpyrophosphate (PRPP) from adenosine triphosphate (ATP) and ribose-5-phosphate (R5P). It is a crucial enzyme in the de novo synthesis and salvage of purines and biosynthesis of pyrimidine and pyrimidine nucleotides (1, 2). PRPS1 is activated by inorganic phosphate and magnesium and can be allosterically inhibited by ADP and purines (2, 3). Human PRPS1 is a 318 amino acid monomer that forms an active hexamer consisting of three homodimers arranged in a propellar-like shape (4). Each homodimer has an active site that binds both ATP and R5P as well as an allosteric inhibitor site. It has been well-established that many different mutations in the PRPS1 gene can lead to disease. Mutations can result in a gain of function with increased expression that leads to excess purine production present in PRS-I superactivity characterized by gout, hearing loss, hypotonia, and ataxia (2, 5). Alternatively, mutations can result in loss of function with decreased expression present in nonsyndromic sensorineural deafness (DFN-2), Charcot-Marie-Tooth disease-5 (CMTX5), and Arts syndrome characterized by sensorineural hearing loss, optic atrophy, ataxia, neuropathy motor development delay and intellectual disability (2,5,6). Mis-regulation of expression and mutation of PRPS1 expression has also been shown to promote proliferation in cancers including neuroblastoma (7), squamous cell carcinoma (8), and acute lymphoblastic leukaemia (9). Additionally, PRPS1 has been shown to be regulated through its phosphorylation state to play a role in DNA repair in the innate immune response (10) and promote tumorigenesis (11).

References:

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