Recombinant Human Purine Nucleoside Phosphorylase/PNP

Catalog Number: 6486-NP

DESCRIPTION

Source	*E. coli*-derived Met1-Ser289, with a C-terminal 6-His tag Accession # P00491
N-terminal Sequence Analysis	Met1
Predicted Molecular Mass	33 kDa

SPECIFICATIONS

SDS-PAGE	30-35 kDa, reducing conditions
Activity	Measured by the phosphorolysis of 7-methyl-6-thioguanosine. The specific activity is >35,000 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	>95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
Formulation	Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Potassium Phosphate, pH 7.4
- Recombinant Human Purine Nucleoside Phosphorylase/PNP (rhPNP) (Catalog # 6486-NP)
- Substrate: 7-Methyl-6-thioguanosine (MESG) (Berry & Associates, Catalog # PR 3790), 10 mM stock in DMSO
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhPNP to 0.2 ng/µL in Assay Buffer.
2. Dilute Substrate to 800 µM in Assay Buffer.
3. Load 50 µL of 0.2 ng/µL rhPNP into the microplate and start the reaction by adding 50 µL of Substrate. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of Substrate.
4. Read at an absorbance of 360 nm in kinetic mode for 5 minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (OD/min) x well volume (L) x } 10^{12} \text{ pmol/mol}}{\text{ext. coeff** } (M^{-1}cm^{-1}) \text{ x path corr.*** (cm) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank
**Using the extinction coefficient 6220 $M^{-1}cm^{-1}$
***Using the path correction 0.320 cm
Note: the output of many spectrophotometers is in mOD

Final Assay Conditions

Per Well:

- rhPNP: 0.010 µg
- Substrate: 400 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

Purine Nucleoside Phosphorylase (PNP) catalyzes the phophorolysis of N-ribosidic bonds of purine nucleosides and deoxynucleosides. Physiological substrates of PNP include inosine, guanosine, and 2'-deoxyguanosine, but not adenosine (1). PNP is expressed in most tissues, with markedly greater expression in lymphoid tissues. Genetic deficiencies of PNP result in severely compromised T-lymphocyte function and neurologic dysfunction (2, 3). PNP is used in assays for the measurement of inorganic phosphate (4).

References:

1. Schramm, V.L. (1998) Annu. Rev. Biochem. **67**:693.
2. Stoop, W. *et al.* (1977) N. Eng. J. Med. **296**:651.
3. Markert, M.L. (1991) Immunodefic. Rev. **3**:45.
4. Webb, M.R. (1992) Proc. Natl. Acad. Sci. USA. **89**:4884.

PRODUCT SPECIFIC NOTICES

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