

DESCRIPTION

Source *E. coli*-derived
Ala2-Gln173, with an N-terminal Met and 7-His tag
Accession # Q93096

N-terminal Sequence Analysis Met

Predicted Molecular Mass 21 kDa

SPECIFICATIONS

SDS-PAGE 24 kDa, reducing conditions

Activity Measured by its ability to cleave a substrate, p-Nitrophenyl phosphate (pNPP).
The specific activity is >0.35 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 >80%, 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 HEPES, NaCl, Betamercaptoethanol and Brij-35. See Certificate of Analysis for details.

Activity Assay Protocol

- Materials**
- Assay Buffer: 50 mM HEPES, 10 mM DTT, pH 7.5
 - Recombinant Human PRL-1/PTP4A1 (rhPTP4A1) (Catalog # 8490-PT)
 - Substrate: p-Nitrophenyl phosphate (Sigma, Catalog # N2765), 10 mM stock in deionized water
 - NaOH, 0.2 M in deionized water
 - 96-well Clear Plate (Catalog # DY990)
 - Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

- Assay**
1. Dilute rhPTP4A1 to 40 μg/mL in Assay buffer.
 2. Dilute Substrate to 5 mM in Assay buffer.
 3. Prepare reaction mixtures by combining equivalent volumes of dilute rhPTP4A1 and dilute Substrate in microtubes. Include an Enzyme Control by combining dilute rhPTP4A1 with twice the volume of 0.2 M NaOH, mix briefly, then add a volume of dilute Substrate equivalent to the volume of rhPTP4A1. The Enzyme Control will have 2x the volume of the reaction mixture.
 4. Incubate Reactions and Enzyme Controls at 37 °C for 24 hours.
 5. Load 100 μL of Reactions into a plate in triplicate and stop the reactions by adding 100 μL 0.2 M NaOH.
 6. Load 200 μL of Enzyme Controls into plate in triplicate.
 7. Read plate at 410 nm (absorbance) in endpoint mode.
 8. Calculate specific activity:

$$\text{Specific Activity (pmol/min/}\mu\text{g)} = \frac{\text{Adjusted Abs}^* (\text{OD}) \times \text{Conversion Factor}^{**} (\text{pmol/OD})}{\text{Incubation time (min)} \times \text{amount of enzyme } (\mu\text{g})}$$

*Adjusted for Enzyme Controls.

**Derived using calibration standard p-Nitrophenol (Sigma, Catalog # 241326).

- Final Assay Conditions**
- Per Well:
- rhPTP4A1: 2 μg
 - pNPP: 1.25 mM

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

Phosphatase of regenerating liver 1 (PRL-1), also known as protein-tyrosine phosphatase 4A1 (PTP4A1), is a member of the PRL subgroup of PTPases (1). It was originally identified as an immediate-early gene in regenerating liver and later shown to be a PTPase that modulates cell growth (2, 3). PRL-1 is widely expressed in human tissues, and the rat enzyme is expressed in neurons and oligodendrocytes of the cerebral cortex, hippocampus, and cerebellum (4, 5). Human PRL-1 shares 100% amino acid sequence identity with mouse and rat PRL-1. The crystal structure of PRL-1 shows that the protein exists as a trimer (6, 7). PRL-1 localizes to the endoplasmic reticulum in interphase cells in a farnesylation-dependent manner, and to centrosomes during mitosis in a farnesylation-independent manner (8, 9). PRL-1 has been implicated in cell migration, invasion, and metastasis, suggesting that it may play a role in cancer (10-13). PRL-1 promotes colorectal cancer cell growth, migration, and invasion *in vitro* and tumor growth *in vivo* (14). Additionally, elevated PRL-1 expression is correlated with tumor progression in human hepatocellular carcinoma (15).

References:

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