R&D SYSTEMS
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Recombinant Human PRL-3/PTP4A3

Catalog Number: 8455-PT

DESCRIPTION

Source	*E. coli*-derived Ala2-Met173, with N-terminal Met and 7-His tag Accession # O75365
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	20 kDa

SPECIFICATIONS

SDS-PAGE	23 kDa, reducing conditions
Activity	Measured by its ability to cleave a substrate, p-Nitrophenyl phosphate (pNPP). The specific activity is >0.4 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	>85%, 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, DTT and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM HEPES, 10 mM DTT, pH 7.5
- Recombinant Human PRL-3/PTP4A3 (rhPTP4A3) (Catalog # 8455-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 rhPTP4A3 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 rhPTP4A3 and dilute Substrate in microtubes. Include an Enzyme Control by combining dilute rhPTP4A3 with twice the volume of 0.2 M NaOH, mix briefly, then add a volume of dilute Substrate equivalent to the volume of rhPTP4A3. 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:

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

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

Final Assay Conditions

Per Well:

- rhPTP4A3: 2 µg
- pNPP: 1.25 mM

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.

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BACKGROUND

Phosphatase of regenerating liver 3 (PRL-3), also known as protein-tyrosine phosphatase 4A3 (PTP4A3), is a member of the PRL subgroup of PTPases (1). It is preferentially expressed in skeletal muscle and the heart (2). Human PRL-3 shares 97% amino acid sequence identity with mouse and rat PRL-3. Structurally, PRL-3 consists of a five-stranded β-sheet and six α-helices (3, 4). It has been shown to associate with membranes in a farnesylation-dependent manner (5). Both PRL-3 over-expression and attenuation of PRL-3 expression results in cell cycle arrest, suggesting that basal expression levels of this enzyme are important for normal cell cycle progression (6). PRL-3 has been shown to activate NF-κB signaling and may itself be regulated by FKBP38 (7, 8). PRL-3 also promotes epithelial to mesenchymal transition, tumor angiogenesis, cell migration, invasion, and metastasis, and it is over-expressed in multiple human cancers (9-18). Src-mediated phosphorylation of PRL-3 may be required for PRL-3-dependent cell migration and invasion (19).

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