PTP1B full length. E.coli Active Enzyme

Background

Protein tyrosine phosphatase 1B (PTP1B) is an enzyme that removes phosphate groups covalently attached to tyrosine residues in proteins. This ubiquitously expressed enzyme is anchored in the endoplasmic reticulum by its C-terminal end and has its catalytic regions exposed to the cytosol. PTP1B will dephosphorylate a wide variety of phosphoproteins, such as receptors for the growth factors insulin and epidermal growth factor (EGF), c-Src and beta-catenin. Of particular interest is the observation that PTP1B knock-out mice are resistant to high-caloric intake-induced obesity and have exaggerated insulin responses, suggesting that PTP1B may play an important role in regulating growth factor responsiveness.

Ordering Information

Catalog Number
X1765E

Size
20 μg

Customer Storage
Product should be stored at -80°C. Aliquot to avoid freeze/thaw cycles

Formulation
Provided in 25 mM Tris-HCl, 75 mM NaCl, pH 8.0, 0.05% Tween, 2 mM EDTA, 1 mM DTT and 50% glycerol, 10 mM glutathione

Ship Conditions
Ship on dry ice, freeze upon arrival

Stability
Products are stable for one year from purchase when stored properly

Concentration
Lot specific, see vial

Source
Full-Length PTP1B (a.a. 1-436) with N-terminal GST-HIs tag expressed in E.coli expression system

Activity

Useful for the study of enzyme kinetics, regulation, to dephosphorylate target substrates and for screening inhibitors. Specific Activity: 10 U/ug. One unit will hydrolyze 1 nmol p-nitrophenyl phosphate per minute at pH 7.4 and 30 °C. Assay buffer: 50 mM HEPES, pH 7.4, 2 mM EDTA, 3mM DTT, 100 mM NaCl, 50 mM pNPP.

Purity

>70% by SDS-PAGE

Applications

Useful for the study of enzyme kinetics, screening inhibitors, and selectivity profiling.

Exalpha Biologicals, Inc. 2 Shaker Road, Bldg. B101 Shirley, MA 01464
Tel: 978.425.1370 www.exalpha.com info@exalpha.com

Assay Methods

Materials

Assay Buffer: 50 mM HEPES, ph 7.4, 100 mM NaCl, 2 mM EDTA, 3 mM DTT
Substrate: 5X pNPP (10 mM) in assay buffer
Enzyme Prep.: Dilute enzyme (0.4 mg/ml starting concentration) 1:80 in assay buffer.

Procedure

1. Prepare reaction mixtures in 96-well plate (keep on ice) in the following order:
- 60 μl of assay buffer
- 20 μl of 10 mM pNPP in assay buffer (final conc. 2 mM)
- 20 μl of 5 ng/μl PTP1B enzyme in assay buffer (final conc. 25 nM).
- Mix well and start reaction at 30°C water bath and incubate for 10 min.
2. Add 100 μl per well of 2 M K_2CO_2 to stop reaction.
3. Read absorbance at 405 nm using a plate reader.

References

1. Frangioni, J.V., et al. (1992). The nontransmembrane tyrosine phophatase PTP-1B localizes to the endoplasmic reticulum via its 35 amino acid C-terminal sequence. Cell 68(3);545-560

2. Elchebly, M., et al (1999). Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science 283(5407);1544-1548

Product Specific References