



## Recombinant PTP1B (1–321)/PTPN1 Active Enzyme

### BACKGROUND

Tyrosine-protein phosphatase, non-receptor type 1 (PTP1B) also known as Protein-tyrosine phosphatase 1B, PTPN1 is a PTP (protein tyrosine phosphatase) first isolated from human placenta. The protein is unrelated to other known phosphatases but is similar to common leukocyte antigen (CD45) and to LAR a homolog of the neural adhesion molecule NCAM. PTP1B negatively regulates insulin sensitivity by dephosphorylating the insulin receptor. Akt is a ser/thr kinase effector of insulin signaling that phosphorylates substrates at the consensus motif RXXXS/T. PTP1B contains the Akt phosphorylation motif (RYRDVS50), and PTP1B (but not mutants with substitutions for Ser50) are phosphorylated by Akt. Insulin stimulation also causes a significant increase in phosphorylation of PTP1B.

### ORDERING INFORMATION

#### CATALOG NUMBER

X1659E

#### SIZE

10  $\mu$ g

#### STORAGE CUSTOMER

Enzyme should be stored at  $-20^{\circ}\text{C}$ . Enzyme should be kept on ice when dispensing

#### STABILITY

Products are stable for one year from purchase when stored properly

#### SHIP CONDITIONS

Ship on gel ice, store at  $-20^{\circ}\text{C}$  immediately upon arrival

#### FORMULATION

Provided in 25 mM Tris-HCl, 75 mM NaCl, pH 8.0, 0.05% Tween, 5 mM DTT and 50% glycerol

#### CONCENTRATION

Varies from lot to lot, see label

#### SOURCE

Recombinant enzyme produced in *E. coli*

### ACTIVITY

20 nmole/min/ $\mu$ g of enzyme; Determined using pNPP; Reaction conditions: 50  $\mu$ M pNPP, 10 min incubation at  $30^{\circ}\text{C}$ , 0.05  $\mu$ g enzyme.

### PURITY

>90% pure as determined by Coomassie-stained SDS gel

### REFERENCES

- [1] Chernoff J., Schievella A.R., Jost C.A., Erikson R.L., Neel B.G.; "Cloning of a cDNA for a major human protein-tyrosine-phosphatase."; Proc. Natl. Acad. Sci. U.S.A. 87:2735–2739(1990).
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- [3] Deloukas P., Matthews L.H., Ashurst J.L., Burton J., Gilbert J.G.R., Jones M., Stavrides G., Almeida J.P., Babbage A.K., Bagguley C.L., Bailey J., Barlow K.F., Bates K.N., Beard L.M., Beare D.M., Beasley O.P., Bird C.P., Blakey S.E., Bridgeman A.M., , Rogers J.; "The DNA sequence and comparative analysis of human chromosome 20."; Nature 414:865–871(2001).
- [4] Strausberg R.L., Feingold E.A., Grouse L.H., Derge J.G., Klausner R.D., Collins F.S., Wagner L., Shenmen C.M., Schuler G.D., Altschul S.F., Zeeberg B., Buetow K.H., Schaefer C.F., Bhat N.K., Hopkins R.F., Jordan H., Moore T., Max S.I., Wang J., , Marra M.A.; "Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences."; Proc. Natl. Acad. Sci. U.S.A. 99:16899–16903(2002).
- [5] Charbonneau H., Tonks N.K., Kumar S., Diltz C.D., Harrylock M., Cool D.E., Krebs E.G., Fischer E. H., Walsh K.A.; "Human placenta protein-tyrosine-phosphatase: amino acid sequence and relationship to a family of receptor-like proteins."; Proc. Natl. Acad. Sci. U.S.A. 86:5252–5256(1989).

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## **ASSAY METHODS**

### **MATERIALS**

1. Assay Buffer: 50 mM HEPES, pH 7.4, 100 mM NaCl, 2 mM EDTA, 3 mM DTT, 0.1% BSA.
2. Stop solution: 2M K<sub>2</sub>CO<sub>3</sub>
3. 190 mM pNPP
4. Microtiter plate (Low binding plates recommended)
5. Microtiter plate reader capable of measurements at 405 nm
6. Water bath or incubator at 30°C

### **PROCEDURE**

1. Dilute enzyme to 0.1  $\mu\text{g}/\mu\text{l}$  in assay buffer
1. Prepare reaction mixture:
  - a. 63  $\mu\text{l}$  assay buffer
  - b. 10  $\mu\text{l}$  1% BSA
  - c. 26  $\mu\text{l}$  pNPP (Final concentration of pNPP is 50 mM)
  - d. 1  $\mu\text{l}$  of PTP1B (0.1  $\mu\text{g}/\mu\text{l}$ )
2. Mix well and start reaction at 30°C in water bath and incubate for 10 min.
3. Add 100  $\mu\text{l}$  per well of 2 M K<sub>2</sub>CO<sub>3</sub> to stop the reaction.
4. Read absorbance at 405 nm using a microtiter plate reader.

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