Recombinant Human TC-PTP (2–315)/PTPN2 Active Enzyme

Background

T-cell protein tyrosine phosphatase (TC-PTP), also known as PTPT and PTPN2, is an enzyme that removes phosphate groups covalently attached to tyrosine residues in proteins. This enzyme has two C-terminal end splice variants with distinctly different subcellular localizations. The shorter 45 kilodalton isoform is exclusively nuclear in resting cells, but redistrubutes to the cytoso upon stimulation with growth factors 1 and cellular stress 2 . The longer 48 kilodalton isoform is exclusively found in the endoplasmic reticulum 3 and seems to have distinctly different physiologic substrates from the smaller isoform. 1, 4 Although found in many cell types and tissues, TC-PTP is particularly prominent in hemopoietic cell types. 5, 6 Knockout mice lacking TC-PTP are born viable but die 3 to 5 weeks after birth of erythropoietic and lymphopoietic deficits 7 , indicating a critical role for TC-PTP in bone marrow maturation. TC-PTP will dephosphorylate a wide range of phosphoproteins, such as p52 Shc 6 and receptors for EGF 1 , Insulin 8 and growth hormone. 6 The recombinant protein lacks the C-terminal 100 amino acids that determine intracellular localization but is fully active. 9

Ordering Information

Catalog Number
X1660E

Size
10 μg

Storage Customer
Product should be stored at -20ºC. Aliquot to avoid freeze/thaw cycles

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

Ship Conditions
Ship on gel ice, freeze 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
0.5 mg/ml

Source
Recombinant enyzme produced in E. coli

Activity

1.5 nmole/min/μg of enzyme; Detemined using pNPP; Reaction conditions: 50 μM pNPP, 10 min incubation at 30ºC, 0.5 μg enzyme.

Purity

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

Assay Methods

Materials

1. Assay Buffer: 50 mM HEPES, pH 7.4, 100 mM NaCl, 2 mM EDTA, 3 mM DTT
2. Stop solution: 2M K_2CO_3
3. 190 mM pNPP
4. Microtiter plate
5. Microtiter plate reader capable of measurements at 405 nm
6. Water bath or incubator at 30ºC

Procedure

1. Prepare reaction mixture:
a. 73 μl assay buffer
b. 26 μl pNPP (Final concentration of pNPP is 50 mM)
c. 1 μl of TCPTP
2. Mix well and start reaction at 30ºC in water bath and incubate for 10 min.
3. Add 100 μl per well of 2 M K_2CO_3 to stop the reaction.
4. Read absorbance at 405 nm using a microtiter plate reader.

REFERENCES

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