Store at -80°C

#8005

SHP-1 Phosphatase

☑5 µg

Cell Signaling TECHNOLOGY®

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New 05/08

Description: Purified recombinant full-length human SHP-1 phosphatase, supplied as a GST fusion protein.

Background: SHP-1 (PTPN6) is a non-receptor protein tyrosine phosphatase that is expressed primarily in hematopoietic cells. The enzyme is composed of two SH2 domains, a tyrosine phosphatase catalytic domain and a carboxy-terminal regulatory domain (1). SHP-1 removes phosphates from target proteins to down regulate several tyrosine kinase regulated pathways. In hematopoietic cells, the N-terminal SH2 domain of SHP-1 binds to tyrosine phosphorylated erythropoietin receptors (EpoR) to negatively regulate hematopoietic growth (2). Over expression of SHP-1 in epithelial cells results in dephosphorylation of the Ros receptor tyrosine kinase and subsequent downregulation of Ros-dependent cell proliferation and transformation (3). Following ligand binding in myeloid cells, SHP-1 associates with IL-3R β chain and down regulates IL-3-induced tyrosine phosphorylation and cell proliferation (4). Because SHP-1 down regulates various proliferation pathways, SHP-1 is considered a potential tumor suppressor and angiogenesis regulator (5,6).

Source/Purification: The GST-phosphatase fusion protein was produced by expressing recombinant human SHP-1 (Met1-Lys597) (GenBank Accession No. NM_080548) with an amino-terminal GST tag in *E. coli*. The protein was purified by one-step affinity chromatography using glutathione-agarose.

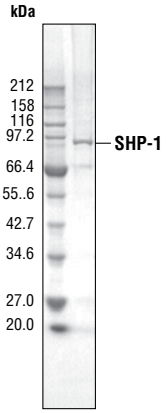

Figure 1. The purity of the GST-SHP-1 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Quality Control: The theoretical molecular weight of the GST-SHP-1 fusion protein is 92 kDa. The purified phosphatase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. SHP-1 phosphatase activity was determined using a DELFIA® assay [Fig.2].

Background References:

(1) Yi, T.L. et al. (1992) *Mol Cell Biol* 12, 836–46.
(2) Yi, T. et al. (1995) *Blood* 85, 87–95.
(3) Keilhack, H. et al. (2001) *J Cell Biol* 152, 325–34.
(4) Yi, T. et al. (1993) *Mol Cell Biol* 13, 7577–86.
(5) Wu, C. et al. (2003) *Gene* 306, 1–12.
(6) Bhattacharya, R. et al. (2008) *J Mol Signal* 3, 8.

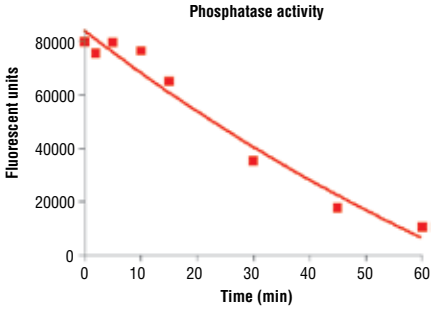

Figure 2. SHP-1 phosphatase activity was measured in a DELFIA® assay using the following reaction conditions: 25 mM HEPES, pH 7.2, 50 mM NaCl, 2.5 mM EDTA, 5 mM DTT, 65 ng/µl BSA, Substrate: Phospho-Poly (Glu-Tyr) Biotinylated Peptide #1586 at 1.5 µM, and 1 ng/µl SHP-1.

Entrez-Gene ID #5777
Swiss-Prot Acc. #P29350

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione.

Store at -80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

Poly (Glu-Tyr) Biotinylated Peptide #1585

Phospho-Poly (Glu-Tyr) Biotinylated Peptide #1586

Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411

Protocol for Tyrosine Phosphatase

Phosphatase Assay:

A Additional Solutions and Reagents (Not included)

- **Phosphatase Buffer (5X)**
  125 mM HEPES, pH 7.2
  250 mM NaCl
  12.5 mM EDTA
- Phospho-Poly (Glu-Tyr) Biotinylated Peptide #1586
- DTT (1.25 M)
- BSA (65 ng/µl)
- Stop solution (2N NaOH)
- Wash Buffer: 1X, PBS 0.05% Tween-20 (PBS/T)
- Phospho-Tyrosine mAb (P-Tyr-100) #9411

B Suggested Protocol for 100 Assays

1. Prepare fresh batches of 1X Phosphatase Assay Buffer by diluting the Phosphatase Buffer (5X) at a 1:4 ratio with a solution containing 5 mM DTT and 65 ng/µl BSA.
2. Dilute 1 mM Phospho-Poly EY (20) Biotinylated Peptide substrate solution to 3 µM with 1X Phosphatase Assay Buffer.
3. Thaw enzyme on ice.
4. Dilute phosphatase protein to 0.2 to 2.0 ng/µl with 1X Phosphatase Assay Buffer.
5. To start the reaction combine 25 µl diluted phosphatase solution and 25 µl substrate (3 µM). Incubate at 37°C for 5 to 60 minutes.

Final Assay Conditions for a 50 µl Reaction

25 mM HEPES, pH 7.2
50 mM NaCl
2.5 mM EDTA
5 mM DTT
65 ng/µl BSA
1.5 µM Phospho-Poly (Glu-Tyr) Biotinylated Peptide
0.1 to 1.0 ng/µl phosphatase

6. Terminate reaction by adding 50 µl of 2N NaOH Stop Solution to each reaction well.
7. For DELFIA® or colorimetric ELISA detection methods please use the protocols described to the right.

DELFIA® Assay

1. Prepare appropriate dilution of Europium labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 µl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 µl/well PBS/T.
5. Add 100 µl/well DELFIA® Enhancement Solution.
6. Incubate at room temperature for 5 minutes.
7. Read plate using a time resolved fluorescent plate reader using the following settings;
   a. Excitation Filter: 340 nm
   b. Emission Filter: 615 nm
   c. Delay++: 400 µs

++ Delay time is the delay from the excitation pulse to the beginning of the measurement.

Companion Products for DELFIA®

DELFIA® Europium-labeled Anti-mouse IgG (PerkinElmer Life Sciences #AD0124)
DELFIA® Europium-labeled Anti-rabbit IgG (PerkinElmer Life Sciences #AD0105)
DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

Colorimetric ELISA Assay

1. Prepare appropriate dilution of HRP labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 µl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 µl/well PBS/T.
5. Add 100 µl/well TMB substrate.
6. Incubate at room temperature for 15 minutes.
7. Add 100 µl/well of stop solution.
8. Mix well.
9. Read the absorbance at 450 nm with a microtiter plate reader.

Companion Products For Colorimetric ELISA Assay

Anti-mouse IgG, HRP Linked Antibody #7076
Anti-rabbit IgG, HRP Linked Antibody #7074
TMB Solution #7004
Stop Solution #7002

*** NOTE:** Use of an automated microplate washer as well as centrifugation of plates when appropriate, greatly improves reproducibility.

For any questions please contact:
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