

Anti-SHP1 Antibody [SR41-02]

ET1602-19



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC
Molecular Wt:	Predicted band size: 68 kDa
Clone number:	SR41-02

Description: Tyrosine-protein phosphatase non-receptor type 6, also known as Src homology region 2 domain-containing phosphatase-1 (SHP-1), is an enzyme that in humans is encoded by the PTPN6 gene. The protein encoded by this gene is a member of the protein tyrosine phosphatase (PTP) family. PTPs are known to be signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation. N-terminal part of this PTP contains two tandem Src homolog (SH2) domains, which act as protein phospho-tyrosine binding domains, and mediate the interaction of this PTP with its substrates. This PTP is expressed primarily in hematopoietic cells, and functions as an important regulator of multiple signaling pathways in hematopoietic cells. This PTP has been shown to interact with, and dephosphorylate a wide spectrum of phospho-proteins involved in hematopoietic cell signaling, (e.g., the LYN-CD22-SHP-1 pathway). Multiple alternatively spliced variants of this gene, which encode distinct isoforms, have been reported.

Immunogen: Synthetic peptide within Human SHP1 aa 537-595 / 595.

Positive control: Jurkat cell lysate, RAW264.7 cell lysate, Mouse spleen tissue lysate, Rat thymus tissue lysate, Jurkat, RAW264.7, human tonsil tissue, mouse spleen tissue, rat brain tissue, rat spleen tissue, mouse brain tissue.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: P29350 Human | P29351 Mouse | P81718 Rat

Recommended Dilutions:

WB	1:2,000-1:5,000
IF-Cell	1:1,000
IF-Tissue	1:50-1:200
IHC-P	1:200-1:1,000
FC	1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4°C. Store at +4°C short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20°C long term.

Purity: Protein A affinity purified.

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Images

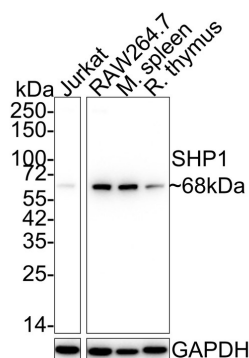


Fig1: Western blot analysis of SHP1 on different lysates with Rabbit anti-SHP1 antibody (ET1602-19) at 1/5,000 dilution.

Lane 1: Jurkat cell lysate (15 µg/Lane)

Lane 2: RAW264.7 cell lysate (15 µg/Lane)

Lane 3: Mouse spleen tissue lysate (20 µg/Lane)

Lane 4: Rat thymus tissue lysate (20 µg/Lane)

Predicted band size: 68 kDa

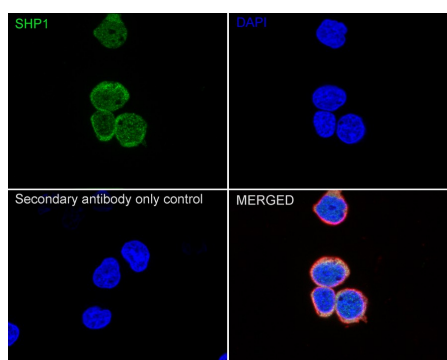
Observed band size: 68 kDa

Exposure time: 24 seconds;

10% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1602-19) at 1/5,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

Fig2: Immunocytochemistry analysis of Jurkat cells labeling SHP1 with Rabbit anti-SHP1 antibody (ET1602-19) at 1/1,000 dilution.



Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-SHP1 antibody (ET1602-19) at 1/1,000 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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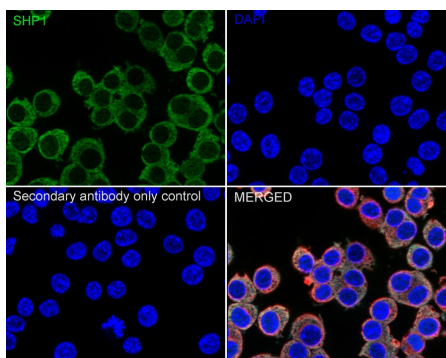
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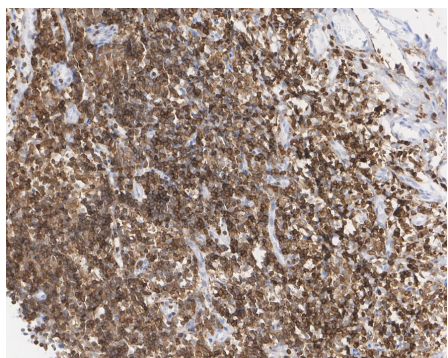
Fig3: Immunocytochemistry analysis of RAW264.7 cells labeling SHP1 with Rabbit anti-SHP1 antibody (ET1602-19) at 1/1,000 dilution.



Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-SHP1 antibody (ET1602-19) at 1/1,000 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

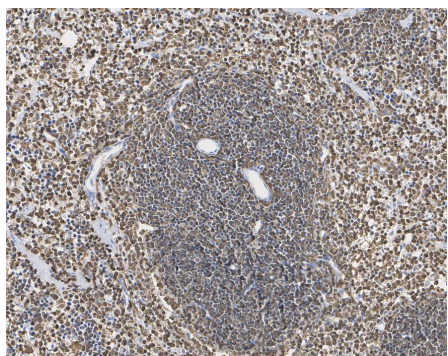
Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Fig4: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-SHP1 antibody (ET1602-19) at 1/1,000 dilution.



The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1602-19) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Fig5: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-SHP1 antibody (ET1602-19) at 1/1,000 dilution.



The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1602-19) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

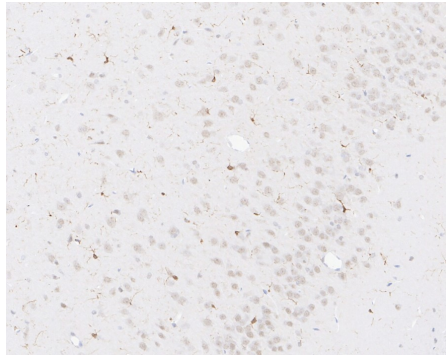


Fig6: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-SHP1 antibody (ET1602-19) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1602-19) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

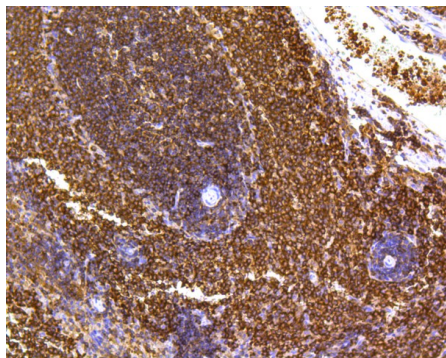


Fig7: Immunohistochemical analysis of paraffin-embedded rat spleen tissue using anti-SHP1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1602-19, 1/200) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

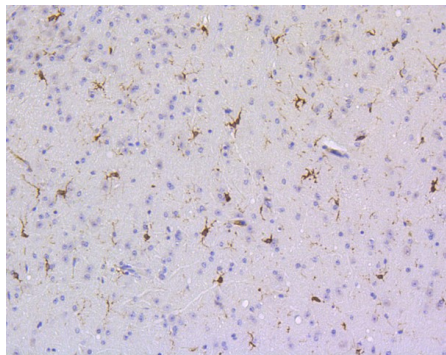


Fig8: Immunohistochemical analysis of paraffin-embedded mouse brain tissue using anti-SHP1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1602-19, 1/200) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

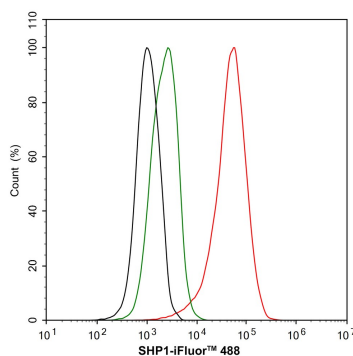


Fig9: Flow cytometric analysis of Jurkat cells labeling SHP1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1602-19, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4 °C for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4 °C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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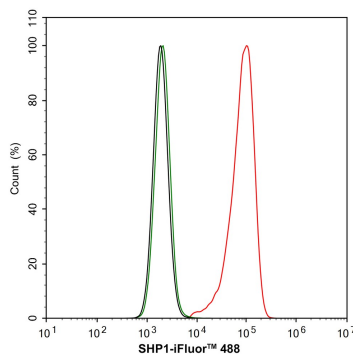


Fig10: Flow cytometric analysis of RAW264.7 cells labeling SHP1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1602-19, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4 °C for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4 °C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Alam HB et al. Alterations in gene expression after induction of profound hypothermia for the treatment of lethal hemorrhage. *J Trauma* 68:1084-98 (2010).
2. Insabato L et al. Elevated Expression of the Tyrosine Phosphatase SHP-1 Defines a Subset of High-Grade Breast Tumors. *Oncology* 77:378-384 (2009).

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