Anti-TRIM21 Antibody [PSH02-56] - BSA and Azide free

HA750819

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 54 kDa
Clone number:	PSH02-56

Description: Tripartite motif-containing protein 21, also known as E3 ubiquitin-protein ligase TRIM21, is a protein that in humans is encoded by the TRIM21 gene. Alternatively spliced transcript variants for this gene have been described but the full-length nature of only one has been determined. It is expressed in most human tissues. TRIM21 is an intracellular antibody effector in the intracellular antibody-mediated proteolysis pathway. It recognizes Fc domain and binds to immunoglobulin G, immunoglobulin A and immunoglobulin M on antibody marked non-enveloped virions which have infected the cell. Either by autoubiquitination or by ubiquitination of a cofactor, it is then responsible for directing the virions to the proteasome. TRIM21 itself is not degraded in the proteasome unlike both the viral capsid and the bound antibody. TRIM21 is part of the RoSSA ribonucleoprotein, which includes a single polypeptide and one of four small RNA molecules. The RoSSA particle localizes to both the cytoplasm and the nucleus.

Immunogen: Recombinant protein within human TRIM21 aa 1-475 (P19474).

Positive control: HeLa cell lysate, HeLa treated with 10ng/mL IFN-α for 16 hours cell lysate, A549 cell lysate, 293T cell lysate, U-2 OS cell lysate, U-2 OS, human spleen tissue, human tonsil tissue.

Subcellular location: Cytoplasm, Cytoplasmic vesicle, autophagosome, Nucleus, P-body.

Database links: SwissProt: P19474 Human

Recommended Dilutions:

WB	1:5,000-1:10,000
IF-Cell	1:100
IHC-P	1:200-1:1,000
FC	1:1,000
IP	1-2μg/sample

Storage Buffer: 1*PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

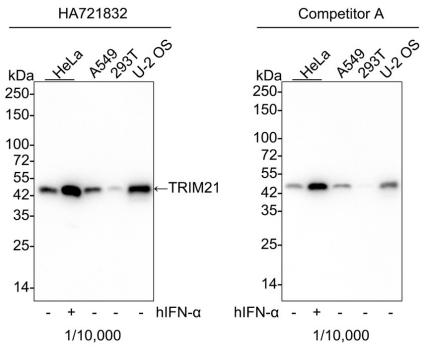

Fig1: Western blot analysis of TRIM21 on different lysates with Rabbit anti-TRIM21 antibody (HA750819) at 1/10,000 dilution and competitor's antibody at 1/10,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 10ng/mL IFN-α for 16 hours cell lysate
Lane 3: A549 cell lysate
Lane 4: 293T cell lysate
Lane 5: U-2 OS cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 54 kDa
Observed band size: 50 kDa

Exposure time: 30 seconds; ECL: K1801;

4-20% 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 (HA750819) at 1/10,000 dilution and competitor's antibody at 1/10,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

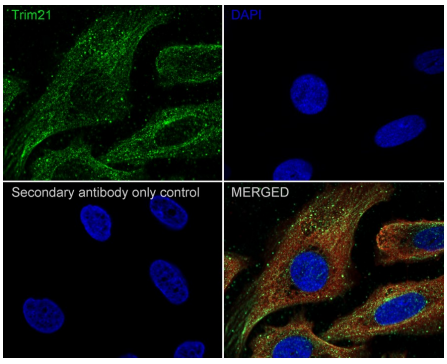

Fig2: Immunocytochemistry analysis of U-2 OS cells labeling TRIM21 with Rabbit anti-TRIM21 antibody (HA750819) at 1/100 dilution.

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-TRIM21 antibody (HA750819) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. 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℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

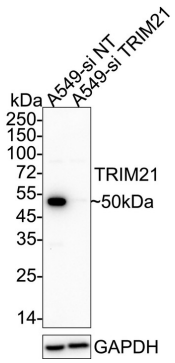

Fig3: Western blot analysis of TRIM21 on different lysates with Rabbit anti-TRIM21 antibody (HA750819) at 1/5,000 dilution.

Lane 1: A549-si NT cell lysate
Lane 2: A549-si TRIM21 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 54 kDa
Observed band size: 50 kDa

Exposure time: 45 seconds; ECL: K1801;

4-20% 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 (HA750819) 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.

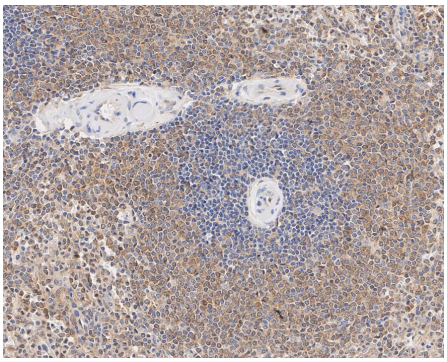

Fig4: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-TRIM21 antibody (HA750819) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750819) 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.

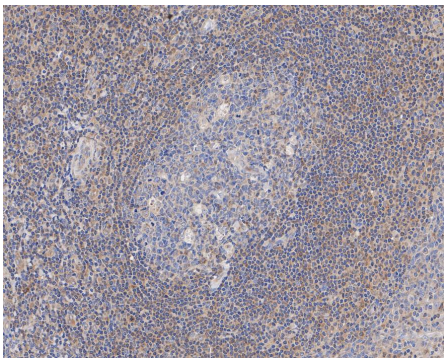

Fig5: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-TRIM21 antibody (HA750819) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750819) at 1/200 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.

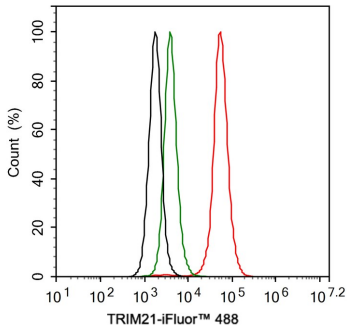

Fig6: Flow cytometric analysis of U-2 OS cells labeling TRIM21.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750819, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ 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℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

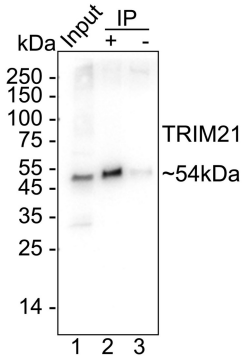

Fig7: TRIM21 was immunoprecipitated from 0.2 mg A549 cell lysate with HA750819 at 2 μg/10 μl beads. Western blot was performed from the immunoprecipitate using HA750819 at 1/1,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: A549 cell lysate (input)
Lane 2: HA750819 IP in A549 cell lysate
Lane 3: Rabbit IgG instead of HA750819 in A549 cell lysate

Blocking/Dilution buffer: primary antibody dilution (K1803)
Exposure time: 6 seconds; ECL: K1801

Background References

1. Gao W et al. TRIM21 regulates pyroptotic cell death by promoting Gasdermin D oligomerization. Cell Death Differ. 2022 Feb
2. Hou K et al. Loss of TRIM21 alleviates cardiotoxicity by suppressing ferroptosis induced by the chemotherapeutic agent doxorubicin. EBioMedicine. 2021 Jul