Anti-WASP Antibody [JE36-82]

HA721659

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, FC
Molecular Wt:	Predicted band size: 53 kDa
Clone number:	JE36-82

Description: The Wiskott–Aldrich Syndrome protein (WASp) is a 502-amino acid protein expressed in cells of the hematopoietic system that in humans is encoded by the WAS gene. In the inactive state, WASp exists in an autoinhibited conformation with sequences near its C-terminus binding to a region near its N-terminus. Its activation is dependent upon CDC42 and PIP2 acting to disrupt this interaction, causing the WASp protein to 'open'. This exposes a domain near the WASp C-terminus that binds to and activates the Arp2/3 complex. Activated Arp2/3 nucleates new F-actin. WASp is the founding member of a gene family which also includes the broadly expressed N-WASP (neuronal Wiskott–Aldrich Syndrome protein), SCAR/WAVE1, WASH, WHAMM, and JMY. WAML (WASP and MIM like), WAWH (WASP without WH1 domain), and WHIMP (WAVE Homology in Membrane Protrusions) have more recently been discovered.

Immunogen: Synthetic peptide within Human WASP aa 1-50 / 502.

Positive control: Jurkat cell lysate, THP-1 cell lysate, Ramos cell lysate, Jurkat, Ramos.

Subcellular location: Cytoplasm, cytoskeleton, Nucleus.

Database links: SwissProt: P42768 Human

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:100
FC	1:500-1:1,000

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

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

Purity: Protein A affinity purified.

Images

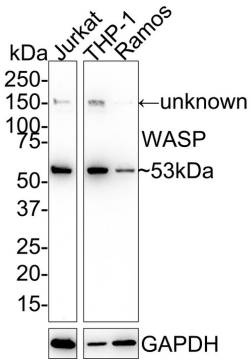

Fig1: Western blot analysis of WASP on different lysates with Rabbit anti-WASP antibody (HA721659) at 1/1,000 dilution.

Lane 1: Jurkat cell lysate
Lane 2: THP-1 cell lysate
Lane 3: Ramos cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 53 kDa
Observed band size: 53 kDa

Exposure time: 1 minute 2 seconds;

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 (HA721659) at 1/1,000 dilution was 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.

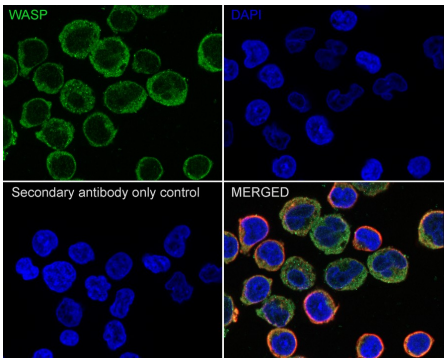

Fig2: Immunocytochemistry analysis of Jurkat cells labeling WASP with Rabbit anti-WASP antibody (HA721659) at 1/100 dilution.

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-WASP antibody (HA721659) 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.

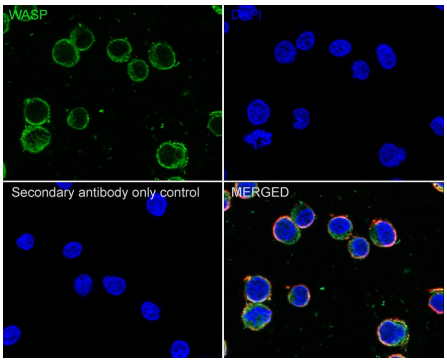

Fig3: Immunocytochemistry analysis of Ramos cells labeling WASP with Rabbit anti-WASP antibody (HA721659) at 1/100 dilution.

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-WASP antibody (HA721659) 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.

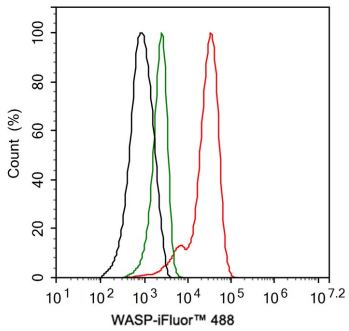

Fig4: Flow cytometric analysis of Jurkat cells labeling WASP.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721659, 1ug/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).

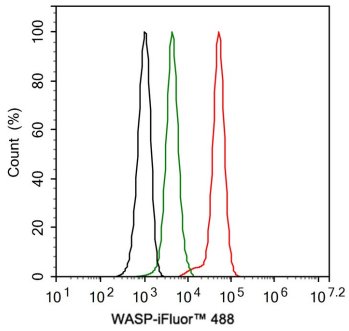

Fig5: Flow cytometric analysis of Ramos cells labeling WASP.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721659, 1ug/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).

Background References

1. Yuan B et al. Wiskott-Aldrich syndrome protein forms nuclear condensates and regulates alternative splicing. Nat Commun. 2022 Jun
2. Sudhakar M et al. Autoimmunity in Wiskott-Aldrich Syndrome: Updated Perspectives. Appl Clin Genet. 2021 Aug