Anti-Survivin Antibody [SP07-06]

ET1604-34

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: 16 kDa
Clone number:	SP07-06

Description: The baculovirus protein p35 inhibits virally-induced apoptosis of invertebrate and mammalian cells and may function to impair the clearing of virally infected cells by the immune system of the host. This is accomplished at least in part by the ability of p35 to block both TNF- and FAS-mediated apoptosis through the inhibition of the ICE family of serine proteases. Two mammalian homologs of baculovirus p35, referred to as inhibitor of apoptosis protein (IAP) 1 and 2, share an amino-terminal baculovirus IAP repeat (BIR) motif and a carboxy-terminal RING finger. Although the c-IAPs do not directly associate with the TNF receptor (TNF-R), they efficiently block TNF-mediated apoptosis through their interaction with the downstream TNF-R effectors, TRAF1 and TRAF2. Additional IAP family members include ILP (for IAP-like protein) and survivin. ILP inhibits activated caspase-3, leading to the resistance of FAS-mediated apoptosis. Survivin (also designated TIAP) is expressed during the G2/M phase of the cell cycle and associates with microtubules of the mitotic spindle. Increased caspase-3 activity is detected when a disruption of survivin-microtubule interactions occurs.

Immunogen: Synthetic peptide within N-terminal human Survivin.

Positive control: Jurkat cell lysate, HeLa cell lysate, 293T cell lysate, RAW264.7 cell lysate, NIH/3T3 cell lysate, C6 cell lysate, PC-12 cell lysate, RAW264.7, HeLa treated with 100ng/mL Nocodazole for 18 hours cell lysate, NIH/3T3 treated with 100ng/mL Nocodazole for 18 hours cell lysate, C6, human tonsil tissue, human lymph node tissue.

Subcellular location: Cytoplasm, Nucleus, Chromosome, Midbody.

Database links: SwissProt: O15392 Human | O70201 Mouse | Q9JHY7 Rat

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:100
IF-Tissue	1:50-1:200
IHC-P	1:200-1:500
FC	1:1,000

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

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

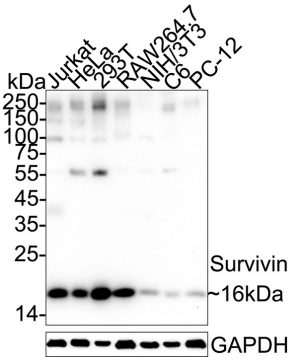

Fig1: Western blot analysis of Survivin on different lysates with Rabbit anti-Survivin antibody (ET1604-34) at 1/1,000 dilution.

Lane 1: Jurkat cell lysate
Lane 2: HeLa cell lysate
Lane 3: 293T cell lysate
Lane 4: RAW264.7 cell lysate
Lane 5: NIH/3T3 cell lysate
Lane 6: C6 cell lysate
Lane 7: PC-12 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 16 kDa
Observed band size: 16 kDa

Exposure time: 2 minutes; 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 (ET1604-34) 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.

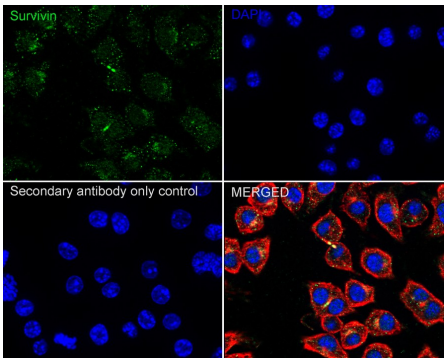

Fig2: Immunocytochemistry analysis of RAW264.7 cells labeling Survivin with Rabbit anti-Survivin antibody (ET1604-34) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-Survivin antibody (ET1604-34) 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 (HA601187, 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.

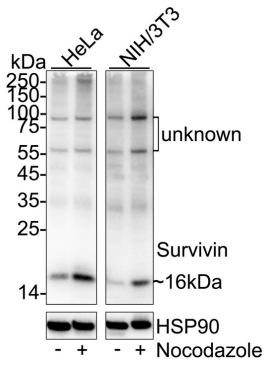

Fig3: Western blot analysis of Survivin on different lysates with Rabbit anti-Survivin antibody (ET1604-34) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 100ng/mL Nocodazole for 18 hours cell lysate
Lane 3: NIH/3T3 cell lysate
Lane 4: NIH/3T3 treated with 100ng/mL Nocodazole for 18 hours cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 16 kDa
Observed band size: 16 kDa

Exposure time: Lane 1-2: 30 seconds; Lane 3-4: 1 minute 2 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 (ET1604-34) at 1/1,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

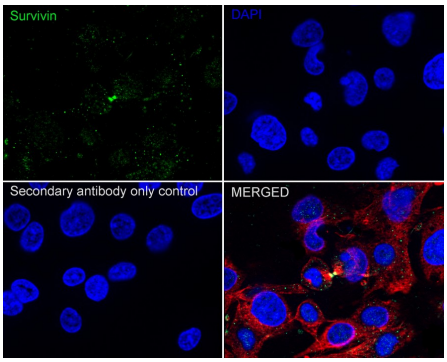

Fig4: Immunocytochemistry analysis of C6 cells labeling Survivin with Rabbit anti-Survivin antibody (ET1604-34) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-Survivin antibody (ET1604-34) 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 (HA601187, 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.

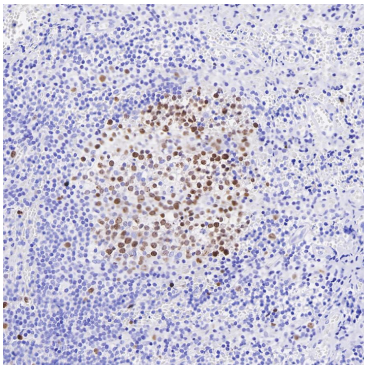

Fig5: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-Survivin antibody (ET1604-34) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (ET1604-34) 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.

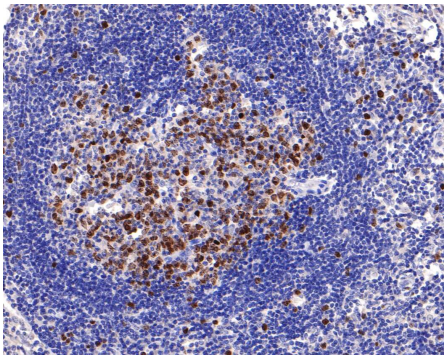

Fig6: Immunohistochemical analysis of paraffin-embedded human lymph node tissue using anti-Survivin antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1604-34, 1/400) 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.

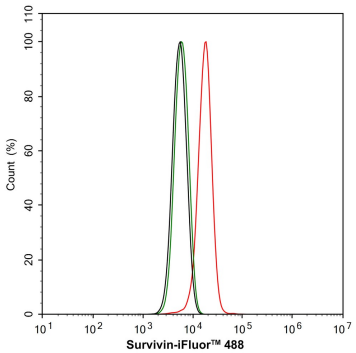

Fig7: Flow cytometric analysis of RAW264.7 cells labeling Survivin.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1604-34, 1/1,000) (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).

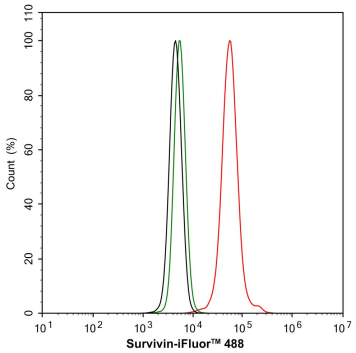

Fig8: Flow cytometric analysis of C6 cells labeling Survivin.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1604-34, 1/1,000) (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. Lin Y et al. Survivin is expressed in degenerated nucleus pulposus cells and is involved in proliferation and the prevention of apoptosis in vitro. Mol Med Rep 13:1026-32 (2016).
2. Cao C et al. The long intergenic noncoding RNA UFC1, a target of MicroRNA 34a, interacts with the mRNA stabilizing protein HuR to increase levels of -catenin in HCC cells. Gastroenterology 148:415-26.e18 (2015).