Anti-Phospho-YAP1 (S127) Antibody [SN0718] ET1611-69

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Tissue
Molecular Wt:	Predicted band size: 54 kDa
Clone number:	SN0718

Description: The Yes-associated protein, otherwise known as YAP, is a 14-3-3-binding molecule that was originally recognized by virtue of its ability to bind to the SH3 domain of Yes. The binding of YAP to 14-3-3 requires the phosphorylation of a homologous serine residue (Ser 112) in the YAP 14-3-3-binding motif. The highly conserved and ubiquitously expressed 14-3-3 proteins regulate differentiation, cell cycle progression and apoptosis by binding intracellular phosphoproteins involved in signal transduction. YAP may link events at the plasma membrane and cytoskeleton to inhibition of transcription in the nucleus in a manner regulated by 14-3-3 proteins. YAP shares homology with the WW domain of TAZ, transcriptional co-activator with PDZ-binding motif, which functions as a transcriptional co-activator by binding to the PPXY motif present in transcription factors. YAP is expressed at high levels in the lung, placenta, prostate, ovary and testis.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser127 of human YAP1.

Positive control: HeLa treated with 100nM Calyculin A for 30 minutes whole cell lysate, NIH/3T3 treated with 100nM Calyculin A for 30 minutes whole cell lysate, C6 treated with 100nM Calyculin A for 30 minutes whole cell lysate, SiHa cell lysates, human kidney tissue, mouse kidney tissue.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: P46937 Human | P46938 Mouse | Q2EJA0 Rat

Recommended Dilutions:

WB	1:5,000
IHC-P	1:50-1:200
IF-Tissue	1:50-1:400

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

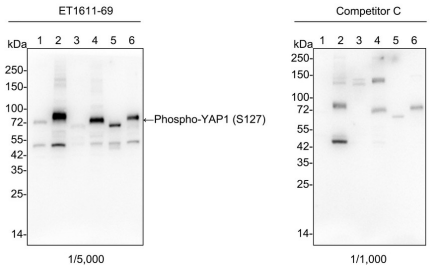

Fig1: Western blot analysis of Phospho-YAP1 (S127) on different lysates with Rabbit anti-Phospho-YAP1 (S127) antibody (ET1611-69) at 1/5,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 100nM Calyculin A for 30 minutes cell lysate
Lane 3: C6 cell lysate
Lane 4: C6 treated with 100nM Calyculin A for 30 minutes cell lysate
Lane 5: NIH/3T3 starved for 24 hours then treated with 100nM Calyculin A for 30 minutes cell lysate
Lane 6: NIH/3T3 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 54 kDa
Observed band size: 70 kDa

Exposure time: 50 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 (ET1611-69) at 1/5,000 dilution and competitor's antibody at 1/1,000 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.

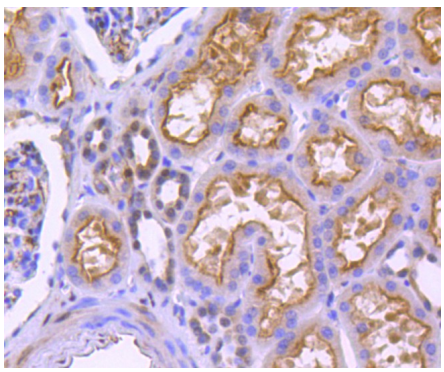

Fig2: Immunohistochemical analysis of paraffin-embedded human kidney tissue using anti-Phospho-YAP1 (S127) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1611-69, 1/50) 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.

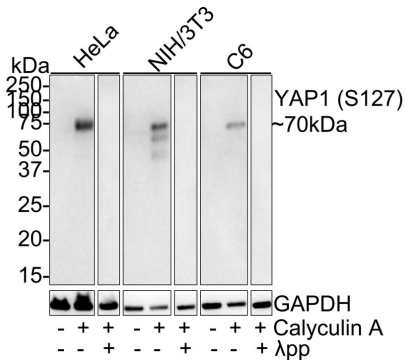

Fig3: Western blot analysis of Phospho-YAP1 (S127) on different lysates with Rabbit anti-Phospho-YAP1 (S127) antibody (ET1611-69) at 1/5,000 dilution.

Lane 1: HeLa whole cell lysate
Lane 2: HeLa treated with 100nM Calyculin A for 30 minutes whole cell lysate
Lane 3: HeLa treated with 100nM Calyculin A for 30 minutes then treated with λpp for 1 hour whole cell lysate
Lane 4: NIH/3T3 whole cell lysate
Lane 5: NIH/3T3 treated with 100nM Calyculin A for 30 minutes whole cell lysate
Lane 6: NIH/3T3 treated with 100nM Calyculin A for 30 minutes then treated with λpp for 1 hour whole cell lysate
Lane 7: C6 whole cell lysate
Lane 8: C6 treated with 100nM Calyculin A for 30 minutes whole cell lysate
Lane 9: C6 treated with 100nM Calyculin A for 30 minutes then treated with λpp for 1 hour whole cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 54 kDa
Observed band size: 70 kDa

Exposure time: 1 minute 6 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 (ET1611-69) 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 1:100,000 dilution was used for 1 hour at room temperature.

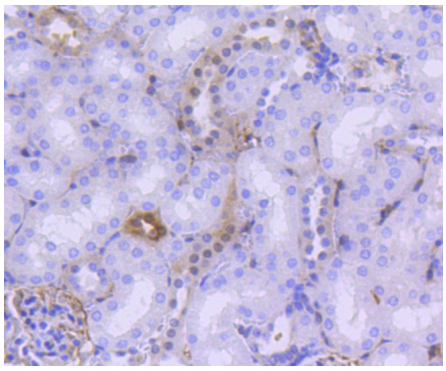

Fig4: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue using anti-Phospho-YAP1 (S127) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1611-69, 1/50) 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.

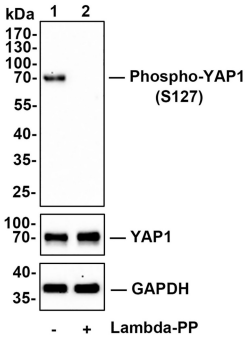

Fig5: Western blot analysis of Phospho-YAP1 (S127) on SiHa cell lysates.

Lane 1: SiHa cells, whole cell lysate, 10ug/lane
Lane 2 : SiHa cells treated with 2.8ug/ul lambda-PP for 30 minutes, whole cell lysates, 10ug/lane

All lanes :
Anti-Phospho-YAP1 (S127) antibody (ET1611-69) at 1/500 dilution. Anti-YAP1 (S127) antibody (ET1608-30) at 1/500 dilution. Anti-GAPDH antibody (ET1601-4) at 1/10,000 dilution. Goat Anti-Rabbit IgG H&L (HRP) (HA1001) at 1/200,000 dilution.

Predicted band size: 54 kDa
Observed band size: 70 kDa

Blocking and diluting buffer: 5% BSA.

Exposure time: 2 minutes 34 seconds

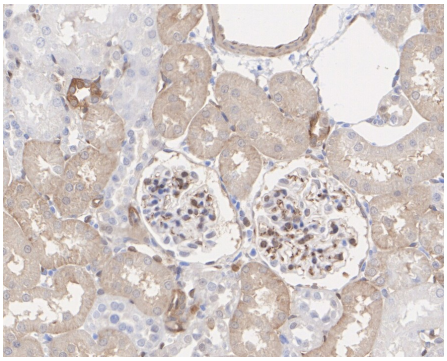

Fig6: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-Phospho-YAP1 (S127) antibody (ET1611-69) at 1/2,000 dilution.

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 (ET1611-69) 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.

Background References

1. Ye L et al. Decreased Yes-Associated Protein-1 (YAP1) Expression in Pediatric Hearts with Ventricular Septal Defects. PLoS One 10:e0139712 (2015).
2. Whiteman EL et al. Crumbs3 is essential for proper epithelial development and viability. Mol Cell Biol 34:43-56 (2014).