Product Datasheet

YAP1 Antibody - BSA Free NB110-58358

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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NB110-58358

YAP1 Antibody - BSA Free	
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	48 kDa
Product Description	
Host	Rabbit
Gene ID	10413
Gene Symbol	YAP1
Species	Human, Mouse, Rat, Canine, Zebrafish
Reactivity Notes	Use in Human reported in scientific literature (PMID:33737385). Use in Zebrafish reported in scientific literature (PMID:28350986).
Specificity/Sensitivity	Expected reactivity based on immunogen homology: Isoform 4 (100%), Isoform 6 (100%)
Immunogen	This YAP1 Antibody was developed against a partial recombinant human YAP1 protein expressed in bacteria. [Uniprot: P46937], N-terminal GST fusion protein
Product Application Details	
Applications	Western Blot, Simple Western, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), Knockdown Validated, Knockout Validated
Recommended Dilutions	Western Blot 1:1000, Simple Western 1:12.5, Immunohistochemistry 1:50-1:200, Immunocytochemistry/ Immunofluorescence, Immunoprecipitation 2-10 ug, Immunohistochemistry-Paraffin 1:50-1:200, Immunohistochemistry-Frozen reported in scientific literature (PMID 28581498), Immunoblotting reported in scientific literature (PMID 28406163), Chromatin Immunoprecipitation (ChIP), Knockout Validated, Knockdown Validated reported in scientific literature (PMID 28406163)
Application Notes	In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See <u>Simple Western Antibody Database</u> for Simple Western validation: Tested in HeLa lysate 0.1 mg/mL, separated by Size, antibody dilution of 1:12.5, apparent MW was 74 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.

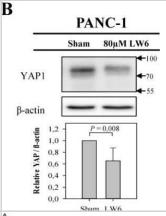


Images

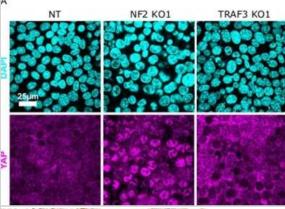
Immunocytochemistry/Immunofluorescence: YAP1 Antibody - BSA Free [NB110-58358] - Caco-2 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with YAP1 Antibody (NB110-58358) at 1 ug/ml overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



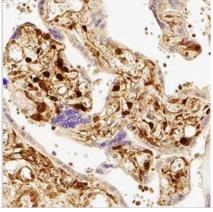
Western Blot: YAP1 Antibody - BSA Free [NB110-58358] - LW6 attenuates the accumulation of cellular YAP1 and its nuclear location. After treating PANC-1 cells with LW6 for 12 hours, LW6 decreased the accumulation of YAP1 when compared to Sham treated cells. n =5 per group. Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/31897243/) licensed under a CC-BY license.

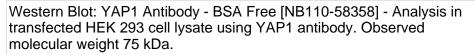


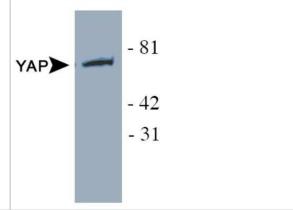
Immunocytochemistry/Immunofluorescence: YAP1 Antibody - BSA Free [NB110-58358] - YAP/TAZ signaling is not activated by loss of TRAF3. NT, NF2 KO1, and TRAF3 KO1 cells stained for YAP1 and DAPI. Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/33185187/) licensed under a CC-BY license.



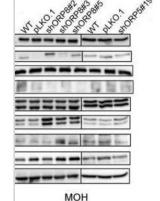
Immunohistochemistry-Paraffin: YAP1 Antibody - BSA Free [NB110-58358] - YAP1 was detected in immersion fixed paraffin-embedded sections of human placenta using Rabbit Anti-Human YAP1 polyclonal Antibody (Catalog # NB110-58358) at 1:200 for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to nuclear and cytoplasm in trophoblast cells.



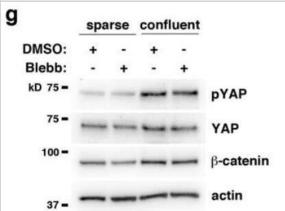




Western Blot: YAP1 Antibody - BSA Free [NB110-58358] - Consequences of ORP5 and ORP8 knockdown on downstream MAPK and PI3K/AKT signaling. Protein from MOH parental, single and double ORP knockdowns as well as cells transfected with empty vector control (pLKO.1) were harvested, and 20 ug was subjected to SDS-PAGE and used for Western blotting. Image collected and cropped by CiteAb from the following publication (https://www.life-science-alliance.org/lookup/doi/10.26508/lsa.201900431), licensed under a CC-BY license.



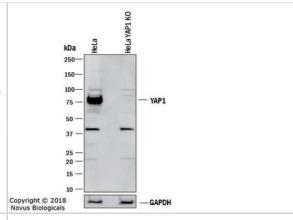
Western Blot: YAP1 Antibody - BSA Free [NB110-58358] - YAP1 Antibody [NB110-58358] - Actomyosin activity inhibits beta-catenin- and YAP-driven proliferation of confluent keratinocytes. Effects of cell density and actomyosin activity on YAP phosphorylation. HaCaT cells cultured for 40 h under the sparse and confluent conditions were treated with 100 uM blebbistatin (Blebb) or DMSO (for control) for 6 h, and then lysed and immunoblotted for Ser127-phosphorylated YAP (pYAP), YAP, beta-catenin and actin. Similar results were obtained in two independent experiments. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/srep46326), licensed under a CC-BY license.



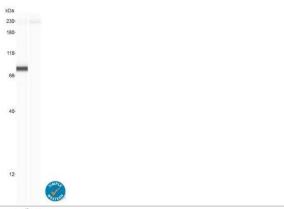
Simple Western: YAP1 Antibody - BSA Free [NB110-58358] - Simple Western lane view shows a specific band for YAP1 in 0.1 mg/ml of HeLa lysate. Observed molecular weight is 75 kDa. This experiment was performed under reducing conditions using the 12-230kDa separation system.



Western Blot: YAP1 Antibody - BSA Free [NB110-58358] - Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and YAP1 knockout (KO) HeLa cell line. PVDF membrane was probed with 1:1000 of Rabbit Anti-Human YAP1 Polyclonal Antibody (Catalog # NB110-58358) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog #HAF008). Specific band was detected for YAP1 at approximately 75 kDa (as indicated) in the parental HeLa cell line, but is not detectable in the knockout HeLa cell line. This experiment was conducted under reducing conditions.

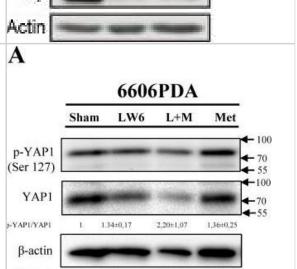


Simple Western: YAP1 Antibody - BSA Free [NB110-58358] - Western lane view shows lysates of HeLa human cervical epithelial carcinoma parental cell line and YAP1 knockout (KO) HeLa cell line. A specific band was detected for YAP1 at approximately 81 kDa (as indicated) using 50 ug/mL of Rabbit Anti-YAP1 Polyclonal Antibody (Catalog # NB110-58358). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



Western Blot: YAP1 Antibody - BSA Free [NB110-58358] - Yap deficiency suppresses cell proliferation in vivo & in vitro. (A-F) The Ki67 positive ratio of lens epithelial cells decreased in Yap-deficient mice at different stages (arrowheads indicate Ki67 positive cells). (G) The relative number of Ki67 positive lens epithelial cells (number of Ki67 positive lens epithelial cells / lens epithelium area). The data are shown as mean ± S.E.M. (Student's t-test, *P<0.05, **P<0.01, n=10). (H-I) Knockdown efficiency of Yap in αTN4 cell using siRNA. (J-K) Cell viability & growth assay revealed that proliferation was downregulated in Yap knockdown α TN4 cells. The data are shown as mean \pm S.E.M. (Two-way RM ANOVA, **P<0.01, n=5). Scale bars: 50 μm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31011480), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

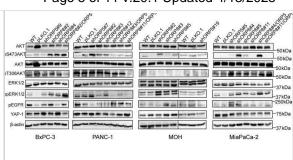
Western Blot: YAP1 Antibody - BSA Free [NB110-58358] - Synergistic effect of LW6 & metformin on YAP1. 80 µM LW6, 20 mM metformin (Met) & the combinational treatment metformin plus LW6 increased phosphorylation of YPA1 at serine 127 & decreased cellular YAP1 concentration after treating cells for 24 hours (A). Moreover, this combinational therapy attenuated the nuclear localization of YAP1 compared to Sham treated cells (B). In addition, lysophosphatidic acid (LPA) & the phosphorylation deficient mutant YAP1-S127A stimulate cell (Ser 127) migration of 6606PDA cells (C & D). n = 2 per group for A, n = 3 per group for B, n = 7 per group for C, n = 9 per group for D. Bar = 5 μ m. Arrows point to nuclei. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31897243), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



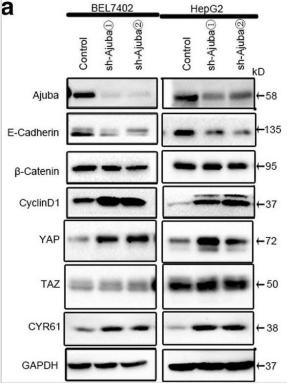


YAPI/B-actin

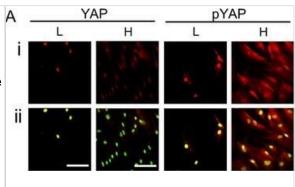
Western Blot: YAP1 Antibody - BSA Free [NB110-58358] - Consequences of ORP5 & ORP8 knockdown on downstream MAPK & PI3K/AKT signaling.Protein from BxPC-3, PANC-1, MiaPaCa-2, & MOH parental, single & double ORP knockdowns as well as cells transfected with empty vector control (pLKO.1) were harvested, & 20 μg was subjected to SDS–PAGE & used for Western blotting. EGFR, MAPK, & PI3K signaling were assayed as pEGFR, ppERK, & pAKT levels, respectively. Amplification of YAP-1 was also evaluated. Total ERK, total AKT, & β-actin levels were used as loading controls. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31451509), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



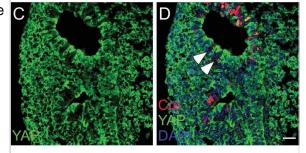
a Western Blot: YAP1 Antibody - BSA Free [NB110-58358] - Ajuba depletion induces β-catenin translocation & Cyclin D1 expression in HCC cell lines. a Immunoblotting with specific antibodies against Ajuba, Ecadherin, β-catenin, Cyclin D1, YAP, TAZ & CYR61 in Ajuba-depleted BEL7402 & HepG2 cells. GAPDH was used as a loading control. The ratios of expression E-Cadherin to their corresponding GAPDH are represented. b Ajuba-depleted HCC cells were fixed for immunofluorescence & stained for β-catenin protein (green) & DAPI (blue). Representative merged images are also shown for fluorescence signals. Scale bar = 25 µm. c Correlation of Ajuba expression with OS in HCC. Low expression of Ajuba was associated with worse OS compared to high expression of Ajuba. Kaplan-Meier curves & log-rank test were used to evaluate OS. P < 0.05 was considered significant. d, e Representative images & quantification of migration & invasion of Ajubadepleted (d) or Ajuba-overexpressing (e) HCC cells. Scale bar = 200 µm. HCC, hepatocellular carcinoma. f Expression in response to the overexpression of constructs of Ajuba-Myc was examined by IB, the ratios of expression Ajuba to their corresponding GAPDH are represented. Data are presented as Mean ± SEM from three independent experiments (*p < 0.05, **p < 0.01, ***p < 0.001) Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30041665), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: YAP1 Antibody - BSA Free A [NB110-58358] - YAP & TAZ expression at high density & during chondrogenic differentiation of human synovial MSCs. (A) YAP & pYAP expression in human synovial membrane-derived (hSM-)MSCs in monolayer at low (L) & high (H) density detected by immunofluorescence staining, shown without (i) & with sytox green nuclear counterstain (ii). (B) YAP & pYAP expression in hSM-MSCs in monolayer at low (L) & high (H) density detected by western blotting with β-actin as loading control. (C, D) Expression of YAP & TAZ (C) & their target genes CTGF & CYR61 (D) in hSM-MSCs immediately prior to (0 h) or 24 h after plating in micromass culture, determined by quantitative RT-PCR. Data was normalised to GAPDH expression, & is shown as mean ± standard deviation (SD) (three donors) relative to pre-seeding (0 h) control. *P <0.05; **P <0.01; ***P <0.001. (E) Expression of YAP & TAZ in hSM-MSCs after 6 days of treatment with 10 ng/ml TGF-β1 or vehicle only in micromass culture to induce chondrogenic differentiation, determined by quantitative RT-PCR. Data was normalised to GAPDH expression, & is shown as mean ± SD (five donors) relative to vehicle-treated control. *P <0.05. (F) Detection of YAP by western blotting during chondrogenic differentiation induced by TGF-β1 with detection of β-actin as loading control. MSC, mesenchymal stromal/stem cell; pYAP, phosphorylated YAP: RT-PCR, reverse transcription PCR: TAZ, transcriptional coactivator with PDZ-binding motif; TGF, transforming growth factor; YAP, Yes-associated protein. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/26025096), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: YAP1 Antibody - BSA Free [NB110-58358] - Expression of spcCre is associated with loss of YAP in SOX9+ distal airways.(A–D) Immunostaining of lung sections collected from Yapf/f; spcCre/+ mice at 13.5 days post coitus (dpc). SOX2 expression marks the proximal airway, while the distal airway is distinguished by SOX9 expression (not shown). High levels of spcCre expression were largely confined to the distal airway, where spcCre expression in a given epithelial cell was correlated with loss of YAP immunoreactivity (e.g. arrowheads in D). (E–H) Immunostaining of lung sections collected from Yapf/f; spcCre/+ mice at 14.5 dpc. Only distal airways are shown. YAP was lost mainly in distal airways in Yapf/f; spcCre/+ mice while sporadic loss of YAP was found in the proximal airway. Loss of YAP was most apparent in the more distal part (arrow in H) of the distal airway, while residual YAP could be found in the more proximal part of the distal airway. Together, these results suggest that lung cyst formation in distal airways of Yapf/f; spcCre/+ mice is due to loss of YAP in the distal airway. Scale bar = 25 µm for A–D; E-H.DOI:http://dx.doi.org/10.7554/eLife.21130.012 Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28323616), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

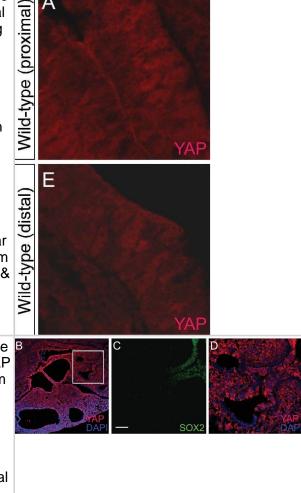


Immunocytochemistry/ Immunofluorescence: YAP1 Antibody - BSA Free [NB110-58358] - The expression patterns of Yap & GFAP-Cre recombinase in postnatal mouse eyes. (A) Schematic of a transverse section of mouse eye. (B-C) Immunostaining with anti-Yap antibody (green) on frozen eye sections at different ages. Nuclei were counterstained with DAPI (blue). Yap staining was detected in scattered cells within the INL (arrowheads) & GCL of the retina & the lens epithelium (arrows). (D) Cre recombinase (red) was expressed in the lens epithelium & INL, GCL of retina in frozen eye sections of Tomatof/+; GFAP-Cre mice at P14. Nuclei were counterstained with DAPI (blue). LE, lens epithelium; TZ, transitional zone; RPE, retinal pigment epithelium; OS, outer segment; IS, inner segment; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Scale bars: 25 µm (B-C), 100 µm (D). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31011480), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

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Immunocytochemistry/ Immunofluorescence: YAP1 Antibody - BSA Free [NB110-58358] - YAP & phospho-YAP are detected in both the proximal & distal airways during lung development.(A-H) Immunostaining of lung sections collected from wild-type mice at 13.5 days post coitus (dpc). The proximal airway is marked by SOX2 expression, while the distal airway is distinguished by SOX9 expression (not shown). Nuclear YAP can be frequently found in both SOX2+ & SOX9+ domains. Similarly, phospho-YAP at S112 (pYAP) could be detected in both the proximal & distal airways, pYAP levels were, in general, higher in the proximal than distal epithelium but pYAP levels varied significantly from cell to cell in both the proximal & distal airways. Representative cells with higher levels of pYAP (arrowhead) are indicated in (B,F). In many cells, low levels of pYAP were associated with the presence of nuclear YAP. This is consistent with a model in which pYAP is sequestered by 14-3-3 proteins in the cytoplasm & degraded but also indicate a dynamic shuttling & distribution of YAP along the entire airway epithelium. Similar results were obtained for lungs collected at 12.5 dpc. Scale bar = 7.5 µm for A-H.DOI:http://dx.doi.org/10.7554/eLife.21130.005 Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28323616), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

Immunocytochemistry/ Immunofluorescence: YAP1 Antibody - BSA Free B [NB110-58358] - Expression of Nkx2.1Cre is associated with loss of YAP in the upper lobes.(A–D) Immunostaining of lung sections collected from Yapf/f; Nkx2.1Cre/+ mice at 14.5 days post coitus (dpc). SOX2 expression marks the proximal airway, while the distal airway is distinguished by SOX9 expression (not shown). YAP was lost mainly in the upper lobe in Yapf/f; Nkx2.1Cre/+ lung. Loss of YAP was more apparent in the distal airway, while loss of YAP was sporadic in the proximal airway. Lung cyst formation was primarily observed in the distal airway. The boxed region in (B) indicates areas shown in (C,D). Scale bar = 250 µm for A,B; 250 µm for C,D. Sox2 expression was present in sporadic Yap-deficient cells in the transition zone induced by Sox9Cre, spcCre or Nkx2.1Cre. This suggests that Sox2 expression is not controlled by YAP.DOI:http://dx.doi.org/10.7554/eLife.21130.013 Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28323616), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

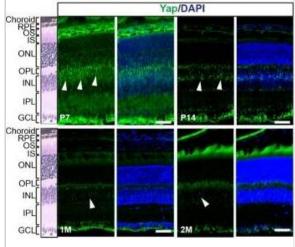


Immunocytochemistry/ Immunofluorescence: YAP1 Antibody - BSA Free [NB110-58358] - Active nuclear YAP is distributed throughout the mouse lung epithelium during development.(A-P) Immunostaining of lung sections collected from wild-type mice at 11.5 & 12.5 days post coitus (dpc). The boxed region in (L) indicates areas shown in (N-P). The proximal airway is marked by SOX2 expression, while the distal airway is distinguished by SOX9 expression. Nuclear YAP can be frequently found in both SOX2+ & SOX9+ domains & is not restricted to the junction (the 'transition zone') between SOX2+ & SOX9+ domains. Representative cells with nuclear YAP (arrowhead) are indicated in (E,P). YAP immunoreactivity is completely absent in the epithelium (but present in the mesenchyme) of Yapf/f; ShhCre/+ mice (M), demonstrating the specificity of YAP antibodies used in this study. Immunofluorescence & immunohistochemistry yielded the same results (data not shown for immunohistochemistry). (Q-R) Whole-mount immunostaining of wildtype & Yap mutant lungs at 11.5 dpc. Distinct domains of SOX2 were discerned in the absence of YAP. Scale bar = 10 µm for A-J; 25 µm for K, L; 10 μm for N–P; 50 μm for Q,

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Immunocytochemistry/ Immunofluorescence: YAP1 Antibody - BSA Free B [NB110-58358] - The expression patterns of Yap & GFAP-Cre recombinase in postnatal mouse eyes. (A) Schematic of a transverse section of mouse eye. (B-C) Immunostaining with anti-Yap antibody (green) on frozen eye sections at different ages. Nuclei were counterstained with DAPI (blue). Yap staining was detected in scattered cells within the INL (arrowheads) & GCL of the retina & the lens epithelium (arrows). (D) Cre recombinase (red) was expressed in the lens epithelium & INL. GCL of retina in frozen eve sections of Tomatof/+: GFAP-Cre mice at P14. Nuclei were counterstained with DAPI (blue). LE, lens epithelium; TZ, transitional zone; RPE, retinal pigment epithelium; OS, outer segment; IS, inner segment; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Scale bars: 25 µm (B-C), 100 µm (D). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31011480), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Schwartzman M, Reginensi A, Wong JS et al. Podocyte-Specific Deletion of Yes-Associated Protein Causes FSGS and Progressive Renal Failure. J. Am. Soc. Nephrol. 2016-01-01 [PMID: 26015453] (Western Blot, Human)

Sunderland A, Williams J, Andreou T et al. Biglycan and reduced glycolysis are associated with breast cancer cell dormancy in the brain Frontiers in Oncology 2023-06-29 [PMID: 37456245] (Western Blot, Human)

Zhao X, Tang L, Le TP et al. Yap and Taz promote osteogenesis and prevent chondrogenesis in neural crest cells in vitro and in vivo Science Signaling 2022-10-25 [PMID: 36282910] (Western Blot, Human)

McCourt JL, Stearns-Reider KM, Mamsa H et al. Multi-omics analysis of sarcospan overexpression in mdx skeletal muscle reveals compensatory remodeling of cytoskeleton-matrix interactions that promote mechanotransduction pathways Skeletal Muscle 2023-01-06 [PMID: 36609344] (Western Blot, Human)

Wolfe AL, Zhou Q, Toska E et al. UDP-glucose pyrophosphorylase 2, a regulator of glycogen synthesis and glycosylation, is critical for pancreatic cancer growth Proceedings of the National Academy of Sciences 2021-08-03 [PMID: 34330832] (Western Blot, Human)

Xiao W, Pahlavanneshan M, Eun CY et al. Matrix stiffness mediates pancreatic cancer chemoresistance through induction of exosome hypersecretion in a cancer associated fibroblasts-tumor organoid biomimetic model Matrix Biology Plus 2022-06-01 [PMID: 35619988] (Western Blot, Human)

Kastan N, Gnedeva K, Alisch T et al. Small-molecule inhibition of Lats kinases may promote Yap-dependent proliferation in postmitotic mammalian tissues Nature Communications 2021-05-25 [PMID: 34035288] (Western Blot, Human)

Sun X, Malandraki-Miller S, Kennedy T et al. The extracellular matrix protein agrin is essential for epicardial epithelial-to-mesenchymal transition during heart development Development 2021-05-01 [PMID: 33969874] (Western Blot, Human)

Moon S, Lee OH, Kim B et al. Estrogen Regulates the Expression and Localization of YAP in the Uterus of Mice International Journal of Molecular Sciences 2022-08-29 [PMID: 36077170] (Western Blot, Human)

Fetiva MC, Liss F, Gertzmann D et al. Oncogenic YAP mediates changes in chromatin accessibility and activity that drive cell cycle gene expression and cell migration Nucleic Acids Research 2023-05-22 [PMID: 36864753] (Western Blot, Human)

Sousa SC, Aroso M, Bessa R, Veríssimo E et Al. Stretch triggers microtubule stabilization and MARCKS-dependent membrane incorporation in the shaft of embryonic axons Curr Biol 2024-09-12 [PMID: 39265571]

Godin P, Tsoi MF, Morin M, Gévry N et Al. The granulosa cell response to luteinizing hormone is partly mediated by YAP1-dependent induction of amphiregulin Cell Commun Signal 2022-05-26 [PMID: 35619099]

More publications at http://www.novusbio.com/NB110-58358



Procedures

Western Blot Protocol for YAP1 Antibody (NB110-58358)

Western Blot Protocol

- 1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
- 2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
- 3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
- 4. Rinse the blot TBS -0.05% Tween 20 (TBST).
- 5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
- 6. Wash the membrane in TBST three times for 10 minutes each.
- 7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
- 8. Wash the membrane in TBST three times for 10 minutes each.
- 9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
- 10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
- 11. Apply the detection reagent of choice in accordance with the manufacturer's instructions.

Immunocytochemistry/ Immunofluorescence Protocol for YAP1 Antibody (NB110-58358) Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

- 1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
- 2. Remove the formalin and wash the cells in PBS.
- 3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
- 4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
- 5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
- 6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
- 7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
- 8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
- 9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
- 10. Counter stain DNA with DAPi if required.





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