abcam

Product datasheet

Anti-YAP1 (phospho S127) antibody [EP1675Y] ab76252

Recombinant RabMAb

★★★★★ 3 Abreviews 79 References 12 Images

Overview

Product name Anti-YAP1 (phospho S127) antibody [EP1675Y]

Description Rabbit monoclonal [EP1675Y] to YAP1 (phospho S127)

Host species Rahhit

Specificity Stimulation may be required to allow detection of the phosphorylated protein. Please see

images belowfor recommended treatment conditions and positive controls.

Tested applications Suitable for: WB, IHC-P, Dot blot

Unsuitable for: ICC/IF

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HeLa, C6 and NIH/3T3 cell lysates treated with Calyculin A. 293A grown in serum-free

media. IHC-P: Human endometrium cancer, Mouse kidney, Mouse stomach, Rat kidney and Rat

stomach tissues. Dot Blot: YAP1 peptides.

General notes This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Stable for 12 months at -20°C.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal
Clone number EP1675Y

Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab76252 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★☆ (3)	1/10000 - 1/50000. Detects a band of approximately 65 kDa (predicted molecular weight: 65 - 75 kDa).
IHC-P		1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
Dot blot		Use at an assay dependent concentration.

Application notes

Is unsuitable for ICC/IF.

Target

Function

Transcriptional regulator which can act both as a coactivator and a corepressor and is the critical downstream regulatory target in the Hippo signaling pathway that plays a pivotal role in organ size control and tumor suppression by restricting proliferation and promoting apoptosis. The core of this pathway is composed of a kinase cascade wherein MST1/MST2, in complex with its regulatory protein SAV1, phosphorylates and activates LATS1/2 in complex with its regulatory protein MOB1, which in turn phosphorylates and inactivates YAP1 oncoprotein and WWTR1/TAZ. Plays a key role to control cell proliferation in response to cell contact. Phosphorylation of YAP1 by LATS1/2 inhibits its translocation into the nucleus to regulate cellular genes important for cell proliferation, cell death, and cell migration. The presence of TEAD transcription factors are required for it to stimulate gene expression, cell growth, anchorage-independent growth, and epithelial mesenchymal transition (EMT) induction. Isoform 2 and isoform 3 can activate the C-terminal fragment (CTF) of ERBB4 (isoform 3).

Tissue specificity

Increased expression seen in some liver and prostate cancers. Isoforms lacking the transactivation domain found in striatal neurons of patients with Huntington disease (at protein level).

Sequence similarities

Belongs to the YORKIE family.
Contains 2 WW domains.

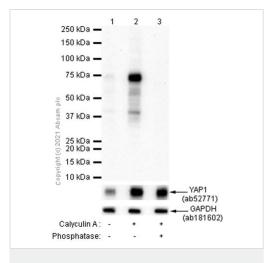
Post-translational modifications

Phosphorylated by LATS1 and LATS2; leading to cytoplasmic translocation and inactivation. Phosphorylated by ABL1; leading to YAP1 stabilization, enhanced interaction with TP73 and recruitment onto proapoptotic genes; in response to DNA damage.

Cellular localization

Cytoplasm. Nucleus. Both phosphorylation and cell density can regulate its subcellular localization. Phosphorylation sequesters it in the cytoplasm by inhibiting its translocation into the nucleus. At low density, predominantly nuclear and is translocated to the cytoplasm at high density.

Images



Western blot - Anti-YAP1 (phospho S127) antibody [EP1675Y] (ab76252) **All lanes :** Anti-YAP1 (phospho S127) antibody [EP1675Y] (ab76252) at 1/1000 dilution

Lane 1 : Untreated HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 15 μg

Lane 2 : HeLa treated with 100ng/ml Calyculin A for 30 min whole cell lysate at 15 μg

Lane 3 : HeLa treated with 100ng/ml Calyculin A for 30 min whole cell lysate, then the membrane treated with Alkaline Phosphatase for 1 hour

Secondary

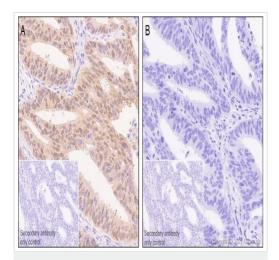
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Developed using the ECL technique.

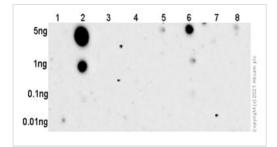
Predicted band size: 65 - 75 kDa **Observed band size:** 75 kDa

Exposure time: 180 seconds

Blocking buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-YAP1 (phospho S127) antibody [EP1675Y] (ab76252)



Dot Blot - Anti-YAP1 (phospho S127) antibody [EP1675Y] (ab76252)

Immunohistochemical analysis of paraffin-embedded sections of human endometrium cancer tissue labelling YAP1 (S127) with ab76252 at 1/500 (0.428 µg/ml) dilution, followed by ready to use secondary antibody Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Counter stained with Hematoxylin.

Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes.

Positive staining on human endometrium cancer without alkaline phosphatase treatment; No signal was detected when tissues were treated with alkaline phosphatase (image B).

The section was incubated with ab76252 for 10 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Dot blot analysis of YAP1 peptides labelling YAP1 (pS127) with ab76252 at 1/1000 dilution (0.24 μ g/ml). **ab97051** (Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated) was used as the secondary antibody at 1/100000 dilution.

Lane 1: YAP1 non-phospho peptide

Lane 2: YAP1 S127 phospho peptide

Lane 3: YAP1 S128 phospho peptide

Lane 4: YAP1 S131 phospho peptide

Lane 5: YAP1 S127+S128 phospho peptide

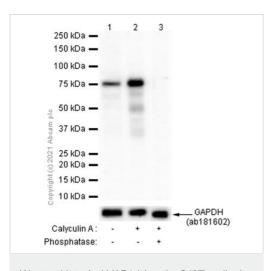
Lane 6: YAP1 S127+S131 phospho peptide

Lane 7: YAP1 S128+S131 phospho peptide

Lane 8: YAP1 S127+S128+S131 phospho peptide

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



Western blot - Anti-YAP1 (phospho S127) antibody [EP1675Y] (ab76252)

All lanes : Anti-YAP1 (phospho S127) antibody [EP1675Y] (ab76252) at 1/1000 dilution

Lane 1 : Untreated NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate at 15 μg

Lane 2 : NIH/3T3 treated with 100ng/ml Calyculin A for 30 min whole cell lysate at 15 μg

Lane 3: NIH/3T3 treated with 100ng/ml Calyculin A for 30 min whole cell lysate, then the membrane treated with Alkaline Phosphatase for 1 hour

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Developed using the ECL technique.

Predicted band size: 65 - 75 kDa **Observed band size:** 75 kDa

Exposure time: 40 seconds

Blocking buffer: 5% NFDM/TBST.

250 kDa — 1 2 3 All lanes 150 kDa — (ab7625)

100 kDa — 75 kDa — 950 kDa — 975 kDa

Western blot - Anti-YAP1 (phospho S127) antibody [EP1675Y] (ab76252)

All lanes : Anti-YAP1 (phospho S127) antibody [EP1675Y] (ab76252) at 1/1000 dilution

Lane 1 : Untreated C6 (Rat glial tumor glial cell) whole cell lysate at $15 \ \mu g$

Lane 2: C6 treated with 100ng/ml Calyculin A for 30 min whole cell lysate at 15 μg

Lane 3 : C6 treated with 100ng/ml Calyculin A for 30 min whole cell lysate, then the membrane treated with Alkaline Phosphatase for 1 hour

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Developed using the ECL technique.

Predicted band size: 65 - 75 kDa

Observed band size: 75 kDa

Exposure time: 60 seconds

Blocking buffer: 5% NFDM/TBST

1 2
250 kDa —
150 kDa —
100 kDa —
75 kDa —
37 kDa —
25 kDa —
20 kDa —
15 kDa —
10 kDa —
11 kDa —
10 kDa —
11 kDa —
11 kDa —
12 kDa —
13 kDa —
14 kDa —
15 kDa —
16 kDa —
17 kDa —
18 kDa —
19 kDa —
19 kDa —
10 kDa —

Western blot - Anti-YAP1 (phospho S127) antibody [EP1675Y] (ab76252)

■ ■ ← GAPDH (ab181602)

All lanes : Anti-YAP1 (phospho S127) antibody [EP1675Y] (ab76252) at 1/1000 dilution

Lane 1 : 293A grown in serum-free media overnight, whole cell lysate

Lane 2: 293A grown in serum-free media overnight, then 10% FBS was added to medium for 1 hour, whole cell lysate

Lysates/proteins at 20 µg per lane.

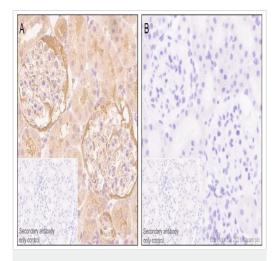
Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

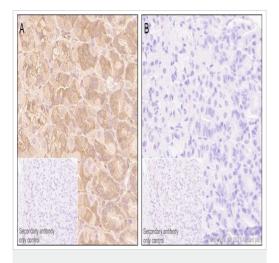
Predicted band size: 65 - 75 kDa **Observed band size:** 75 kDa

Exposure time: 3 minutes

Blocking and dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-YAP1 (phospho S127) antibody [EP1675Y] (ab76252)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-YAP1 (phospho S127) antibody [EP1675Y] (ab76252)

Immunohistochemical analysis of paraffin-embedded sections of rat kidney tissue labelling YAP1 (S127) with ab76252 at 1/500 (0.428 μ g/ml) dilution, followed by ready to use secondary antibody Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>). Counter stained with Hematoxylin.

Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes.

Positive staining on rat kidney without alkaline phosphatase treatment; No signal was detected when tissues were treated with alkaline phosphatase (image B).

The section was incubated with ab76252 for 10 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

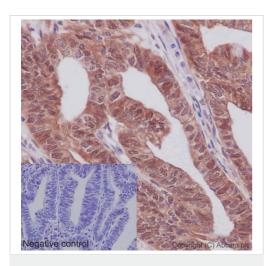
Immunohistochemical analysis of paraffin-embedded sections of mouse stomach tissue labelling YAP1 (S127) with ab76252 at 1/500 (0.428 µg/ml) dilution, followed by ready to use secondary antibody Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Counter stained with Hematoxylin.

Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes.

Positive staining on mouse stomach without alkaline phosphatase treatment; No signal was detected when tissues were treated with alkaline phosphatase (image B).

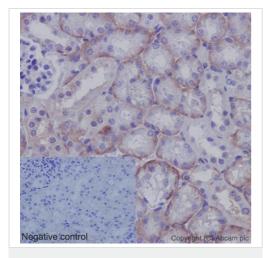
The section was incubated with ab76252 for 10 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



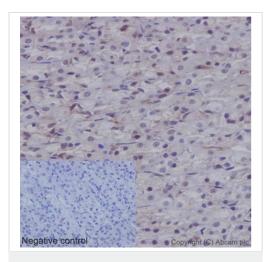
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-YAP1 (phospho S127) antibody [EP1675Y] (ab76252)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human adenocarcinoma of endometrium tissue labelling YAP1 (phospho S127) with purified ab76252 at 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated antirabbit lgG was used as the secondary antibody. Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



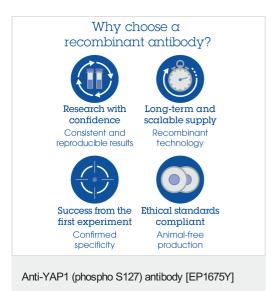
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-YAP1 (phospho S127) antibody [EP1675Y] (ab76252)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse kidney tissue labelling YAP1 (phospho S127) with purified ab76252 at 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit lgG was used as the secondary antibody. Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-YAP1 (phospho S127) antibody [EP1675Y] (ab76252)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat stomach tissue labelling YAP1 (phospho S127) with purified ab76252 at 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit lgG was used as the secondary antibody. Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



(ab76252)

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