# abcam

# Product datasheet

# Anti-YAP1 antibody [EP1674Y] ab52771

יולצעבע RabMAb

★★★★★ 16 Abreviews 217 References 画像数 12

#### 製品の概要

製品名 Anti-YAP1 antibody [EP1674Y]

製品の詳細 Rabbit monoclonal [EP1674Y] to YAP1

由来種 Rabbit

アプリケーション 適用あり: Flow Cyt (Intra), WB, IP, IHC-P, ICC/IF

種交差性 交差種: Human

免疫原 Synthetic peptide within Human YAP1 aa 400-500 (C terminal). The exact sequence is

proprietary.

Database link: P46937

(Peptide available as ab173007)

ポジティブ・コントロール WB: HeLa whole cell lysate; Human thyroid cancer lysates. IHC-P: Human breast carcinoma and

thyroid carcinoma tissue. ICC/IF: MCF7 cells. IP: YAP1 in HeLa whole cell lysate. Flow Cyt (intra):

HeLa cells.

特記事項 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**.

Rat: We have preliminary internal testing data to indicate this antibody may not react with this

species. Please contact us for more information.

製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

バッファー pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA

精製度 Protein A purified

**ポリ/モノ** モノクローナル

**クローン名** EP1674Y

アイソタイプ lgG

#### アプリケーション

**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab52771の使用に適用されますアプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/20.  ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody. For unpurified use at 1:50.
WB	<b>★★★★</b> ★ ★ (5)	1/5000 - 1/50000. Detects a band of approximately 72 kDa (predicted molecular weight: 65 kDa).
IP	**** <u>(3)</u>	1/20. For unpurified use at 1:70.
ІНС-Р	★★★★☆ (3)	1/50. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF	<b>★★★★★</b> ( <u>5</u> )	1/100 - 1/500.

#### ターゲット情報

機能

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).

組織特異性

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).

配列類似性

Belongs to the YORKIE family. Contains 2 WW domains.

翻訳後修飾

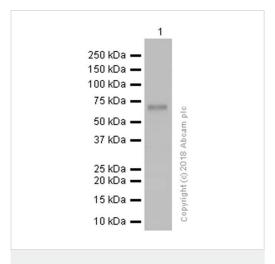
Phosphorylated by LATS1 and LATS2; leading to cytoplasmic translocation and inactivation.

## 細胞内局在

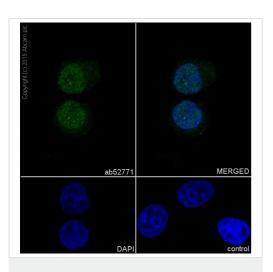
Phosphorylated by ABL1; leading to YAP1 stabilization, enhanced interaction with TP73 and recruitment onto proapoptotic genes; in response to DNA damage.

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.

#### 画像



Western blot - Anti-YAP1 antibody [EP1674Y] (ab52771)



Immunocytochemistry/ Immunofluorescence - Anti-YAP1 antibody [EP1674Y] (ab52771) Anti-YAP1 antibody [EP1674Y] (ab52771) at 1/5000 dilution (purified) + HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates at 15  $\mu$ g

# **Secondary**

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

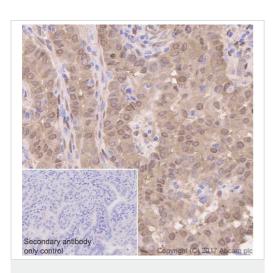
**Predicted band size:** 65 kDa **Observed band size:** 72 kDa

Blocking/Diluting buffer: 5% NFDM/TBST

Immunocytochemistry/Immunofluorescence analysis of MCF-7 (Human breast adenocarcinoma cell line) cells labeling YAP1 with purified ab52771 at 1/500.

Cells were fixed with 4% paraformaldehyde and permeabilized by 0.1% Triton X-100. An Alexa Fluor<sup>®</sup> 488-conjugated goat anti-rabbit lgG (1/1000) was used as the secondary antibody (ab150077).

Nuclei counterstained with DAPI (blue).

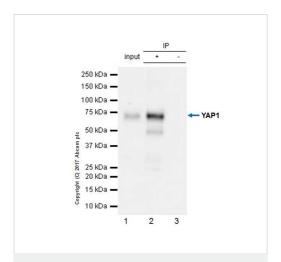


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-YAP1 antibody
[EP1674Y] (ab52771)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human thyroid carcinoma tissue sections labeling YAP1 with purified ab52771 at 1:50 dilution (1.78 µg/ml).

Heat mediated antigen retrieval was performed using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody.

PBS instead of the primary antibody was used as the negative control.



Immunoprecipitation - Anti-YAP1 antibody [EP1674Y] (ab52771)

ab52771 (purified) at 1:20 dilution (0.5  $\mu$ g) immunoprecipitating YAP1 in HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate.

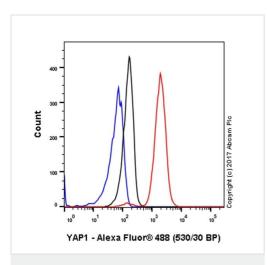
Lane 1: HeLa whole cell lysate 10 µg input.

Lane 2: ab52771 in HeLa whole cell lysate

**Lane 3:** Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab52771 in HeLa whole cell lysate.

For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at 1:1000 dilution.

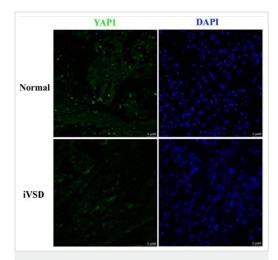
Blocking/Diluting buffer: 5% NFDM/TBST.



Flow Cytometry (Intracellular) - Anti-YAP1 antibody [EP1674Y] (ab52771)

Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling YAP1 with purified ab52771 at 1/20 dilution (10µg/ml) (**red**).

Cells were fixed with 4% paraformaldehyde. A Goat anti rabbit lgG (Alexa Fluorr<sup>®</sup> 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal lgG (**Black**). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (**Blue**).



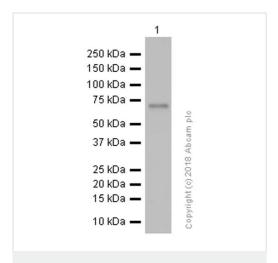
Immunocytochemistry/ Immunofluorescence - Anti-YAP1 antibody [EP1674Y] (ab52771)

Ye et al PLoS One. 2015 Oct 1;10(10):e0139712. doi: 10.1371/journal.pone.0139712. eCollection 2015. Fig 2. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

# Immunofluorescence revealed that YAP1 expression was significantly lower in iVSD hearts compared to control hearts.

YAP1 (green), and DAPI (blue) staining are shown; Scale bar =  $25 \mu m$ .

Cryosections of human heart tissue were blocked using 10% FBS for 30 min, and then incubated with anti YAP1 (ab52771, 1:200) at room temperature for 2 h. The slides were then incubated with the following secondary antibody, Alexa Fluor<sup>®</sup> 488-conjugated antirabbit (Abcam, <u>ab150073</u>; 1:1,000 dilution). Cell nuclei were stained with DAPI.



Western blot - Anti-YAP1 antibody [EP1674Y] (ab52771)

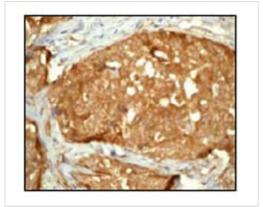
Anti-YAP1 antibody [EP1674Y] (ab52771) at 1/50000 dilution (purified) + Human thyroid cancer lysates at 15  $\mu g$ 

# **Secondary**

Goat Anti-Rabbit  $\lg G$  (HRP) with minimal cross-reactivity with human  $\lg G$  at 1/2000 dilution

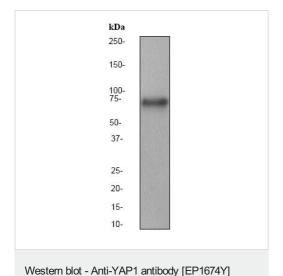
**Predicted band size:** 65 kDa **Observed band size:** 72 kDa

Blocking/Dilution buffer: 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-YAP1 antibody
[EP1674Y] (ab52771)

Immunohistochemical staining of YAP1 in paraffin embedded human breast carcinoma tissue using unpurified ab52771 at a 1/100 dilution.

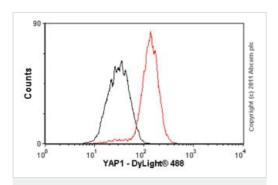


Anti-YAP1 antibody [EP1674Y] (ab52771) at 1/50000 dilution (unpurified) + HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate at 10 µg

## **Secondary**

Goat anti-rabbit HRP at 1/2000 dilution

**Predicted band size:** 65 kDa **Observed band size:** 72 kDa



(ab52771)

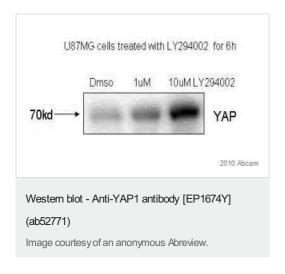
Flow Cytometry (Intracellular) - Anti-YAP1 antibody [EP1674Y] (ab52771)

Overlay histogram showing HeLa (Human epithelial cell line from cervix adenocarcinoma) cells stained with unpurified ab52771 (**red line**).

The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab52771, 1/50 dilution) for 30 min at 22°C. The secondary antibody used was DyLight<sup>®</sup> 488 goat anti-rabbit lgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C.

Isotype control antibody (**black line**) was rabbit IgG (monoclonal) (1  $\mu$ g/1x10<sup>6</sup> cells) used under the same conditions.

Acquisition of >5,000 events was performed.

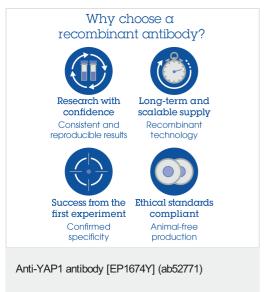


All lanes are unpurified ab52771 at a 1/1000 dilution plus whole cell lysate prepared from U-87 MG (Human glioblastoma-astrocytoma epithelial cell line) cells, treated with DMSO or 1  $\mu$ M and 10  $\mu$ M LY294002, 50  $\mu$ g positive control loaded.

Primary antibody was incubated for 16 hours at 4°C.

Blocking step was performed using 5% milk for 1 hour at 23°C.

Secondary used was a goat polyclonal conjugated to HRP, at a 1/10,000 dilution.



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