# abcam

### Product datasheet

# Anti-active YAP1 antibody [EPR19812] ab205270





重组 RabMAb

★★★★★ 25 Abreviews 70 References 16 图像

概述

产品名称 Anti-active YAP1抗体[EPR19812]

描述 兔单克隆抗体[EPR19812] to active YAP1

宿主 Rabbit

特异性 ab205270 is specific to the active (non-phosphorylated) form of YAP1.

经测试应用 适用于: ICC/IF, IHC-P, WB 种属反应性 与反应: Mouse, Human

免疫原 This product was produced with the following immunogens:

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

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阳性对照 WB: 293A cell lysate serum starved overnight, then 10% FBS was added to medium for 1 hour;

> 293A cell lysate serum starved overnight and then treated with Lambda phosphatase lysate. Human kidney and skin lysates and mouse testis and skin lysates. HaCaT whole cell lysate, 293A cell lysate. HeLa and NIH/3T3 treated with Calyculin A. IHC-P: Human breast and breast cancer tissues; Mouse skin tissue. ICC/IF: 293A cell line serum starved overnight, then 10% FBS was

added to medium for 1 hour; 293A cells.

This product is a recombinant monoclonal antibody, which offers several advantages including: 常规说明

- 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**.

性能

形式 Liquid

存放说明 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.

**存储溶液** pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

纯**度** Protein A purified

**克隆** 单克隆

**克隆编号** EPR19812

同种型 lgG

#### 应用

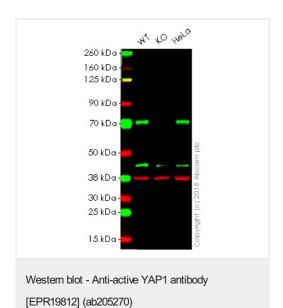
#### The Abpromise guarantee Abpromise™承诺保证使用ab205270于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ICC/IF	**** <u>(2)</u>	1/500.
IHC-P	**** (11)	1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	*** <u>*</u> (2)	1/1000. Detects a band of approximately 75 kDa (predicted molecular weight: 54 kDa).

#### 靶标

#### 图片



All lanes : Anti-active YAP1 antibody [EPR19812] (ab205270) at 1  $\mu$ g/ml

Lane 1: Wild-type HAP1 whole cell lysate

Lane 2 : YAP1 knockout HAP1 whole cell lysate

Lane 3: HeLa whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 54 kDa

**Lanes 1 - 3:** Merged signal (red and green). Green - ab205270 observed at 54 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab205270 was shown to recognize active YAP1 in wild-type HAP1 cells as signal was lost at the expected MW in active YAP1 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and active YAP1 knockout samples were subjected to SDS-PAGE. Ab205270 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1 µg/ml and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

Secondary antibody only control . Copyright (C) 2016 Abcam plc

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-active YAP1 antibody
[EPR19812] (ab205270)

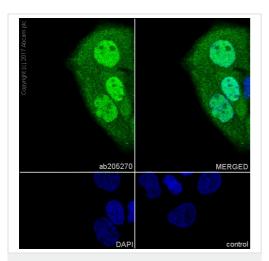
Immunohistochemical analysis of paraffin-embedded human breast tissue labeling active YAP1 with ab205270 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Mainly nuclear staining on human breast is observed [PMID: 18617895].

Counter stained with Hematoxylin.

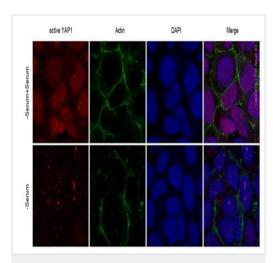
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

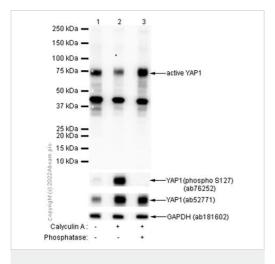


Immunocytochemistry/ Immunofluorescence - Antiactive YAP1 antibody [EPR19812] (ab205270)

Ab205270 staining active YAP1 in HUVEC (human umbilical vein endothelial cell) cells by Immunocytochemistry/Immunofluorescence (ICC/IF). The cells were fixed 4% Paraformaldehyde and permeabilized with 0.1% TritonX-100. Samples were incubated with primary antibody at 1:500 dilution. An Alexa Fluor<sup>®</sup> 488 Goat anti-Rabbit was used as a secondary antibody at 1:1000 dilution. DAPI was used as a nuclear counter stain. Confocal image showing nuclear and cytoplasmic staining in HUVEC cells.



Immunocytochemistry/ Immunofluorescence - Antiactive YAP1 antibody [EPR19812] (ab205270)



Western blot - Anti-active YAP1 antibody [EPR19812] (ab205270)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton/PBS permeabilized (RT, 5 mins) 293A (Human epithelial cell line from embryonic kidney transformed with sheared human adenovirus type 5 DNA) cells labeling active YAP1 with ab205270 at 1/500 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 594) secondary antibody at 1/1000 dilution.

The images showed weak staining on 293A cells under serum starvation overnight. After 10% FBS was added to the medium for 1h, the nuclear staining was increased.

The data was kindly provided by our collaborator Dr. Bin Zhao (Zhejiang University).

The nuclear counterstain is DAPI (blue). Counterstained with Phalloidin-technology<sup>®</sup> Alexa Fluor 488 at 1/1000 dilution.

**All lanes :** Anti-active YAP1 antibody [EPR19812] (ab205270) at 1/1000 dilution

**Lane 1 :** Untreated HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 15  $\mu g$ 

**Lane 2 :** HeLa treated with 100ng/ml Calyculin A for 30 min whole cell lysate at 15  $\mu$ g

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

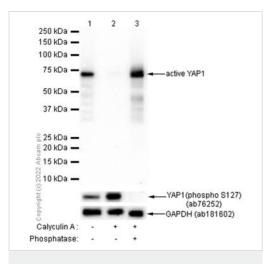
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Developed using the ECL technique.

**Predicted band size:** 54 kDa **Observed band size:** 75 kDa

Exposure time: 80 seconds

Blocking buffer: 5% NFDM/TBST.



Western blot - Anti-active YAP1 antibody [EPR19812] (ab205270)

**All lanes :** Anti-active YAP1 antibody [EPR19812] (ab205270) at 1/1000 dilution

Lane 1 : Untreated NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate at 15  $\mu g$ 

**Lane 2 :** NIH/3T3 treated with 100ng/ml Calyculin A for 30 min whole cell lysate at 15  $\mu$ g

**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 lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Developed using the ECL technique.

Predicted band size: 54 kDa Observed band size: 75 kDa

Exposure time: 20 seconds

Blocking buffer: 5% NFDM/TBST.

**All lanes :** Anti-active YAP1 antibody [EPR19812] (ab205270) at 1/1000 dilution

Lane 1: 293A cells under serum starvation overnight lysateLane 2: 293A cell lysate under serum starvation overnight, then10% FBS was added to medium for 1 hour

Lysates/proteins at 20 µg per lane.

#### 

Western blot - Anti-active YAP1 antibody [EPR19812] (ab205270)

2

250 kDa —

100 kDa — 75 kDa —

#### Secondary

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

**Predicted band size:** 54 kDa **Observed band size:** 75 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

Serum starvation induces active YAP1 Ser127 phosphorylation. The level of active YAP1 protein is inversely proportional to p-YAP1

Ser127 level (PMID: 22884261).

All lanes: Anti-active YAP1 antibody [EPR19812] (ab205270) at 1/1000 dilution

Lane 1: Human kidney lysate

Lane 2: Human skin lysate

Lane 3: HaCaT (Human keratinocyte cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

2 3 250 kDa -250 kDa -250 kDa -150 kDa -150 kDa -150 kDa -100 kDa -100 kDa -100 kDa 🕳 75 kDa 🕳 75 kDa 🕳 75 kDa 🕳 50 kDa -50 kDa -50 kDa -Copyright (c) 2016 Abcam plo 37 kDa -37 kDa -37 kDa -25 kDa -25 kDa 🕳 25 kDa -20 kDa 🕳 20 kDa -20 kDa -15 kDa 🕳 15 kDa -15 kDa 🕳 10 kDa -10 kDa 🕳 10 kDa 🕳

Western blot - Anti-active YAP1 antibody [EPR19812] (ab205270)

# Secondary

Lanes 1-2: Goat Anti-Rabbit IgG Peroxidase Conjugate, specific

to the non-reduced form of IgG at 1/10000 dilution

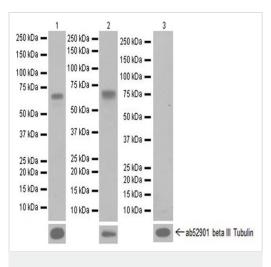
Lane 3: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000

dilution

Predicted band size: 54 kDa Observed band size: 75 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1/2: 8 seconds; Lane 3: 30 seconds.



Western blot - Anti-active YAP1 antibody [EPR19812] (ab205270) **All lanes :** Anti-active YAP1 antibody [EPR19812] (ab205270) at 1/1000 dilution

Lane 1 : Mouse testis lysate
Lane 2 : Mouse skin lysate
Lane 3 : Mouse liver lysate

Lysates/proteins at 10 µg per lane.

#### Secondary

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

**Predicted band size:** 54 kDa **Observed band size:** 75 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1: 10 seconds; Lane 2: 8 seconds; Lane 3: 3

minutes.

**All lanes :** Anti-active YAP1 antibody [EPR19812] (ab205270) at 1/1000 dilution

Lane 1: 293 A cell lysate serum starved overnight and then 10%

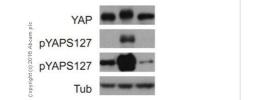
FBS added to the medium for 1 hour

Lane 2: 293A cell lysate serum starved overnight

Lane 3: 293A cell lysate serum starved overnight and then treated

with Lambda phosphatase

Lysates/proteins at 10 µg per lane.



2 3

←130kd

←100kd

←70kd

<-- 55kd

40kd

Western blot - Anti-active YAP1 antibody [EPR19812] (ab205270)

active YAP

#### **Secondary**

**All lanes :** donkey anti-rabbit at 1/1000 dilution

**Predicted band size:** 54 kDa **Observed band size:** 75 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% BSA/TBST.

The two panels for pYAPS127 were just shorter and longer exposure.

The data was kindly provided by our collaborator Dr. Bin Zhao (Zhejiang University).

**All lanes :** Anti-active YAP1 antibody [EPR19812] (ab205270) at 1/1000 dilution

Lane 1: 293A cell lysate

Lane 2: YAP/TAZ knockout 293A cell lysate

Lysates/proteins at 20 µg per lane.

**Secondary** 

All lanes: donkey anti-rabbit at 1/1000 dilution

**Predicted band size:** 54 kDa **Observed band size:** 75 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% BSA/TBST.

The data was kindly provided by our collaborator Dr. Bin Zhao (Zhejiang University).

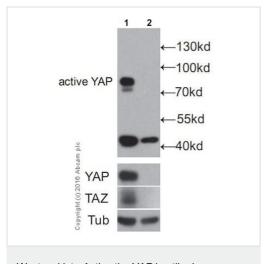
Immunohistochemical analysis of paraffin-embedded human breast cancer tissue labeling active YAP1 with ab205270 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Nuclear and cytoplasmic staining on human breast cancer is observed [PMID: 24559095].

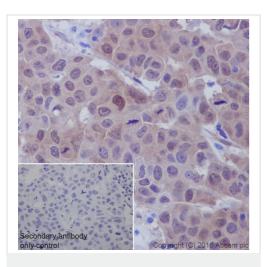
Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

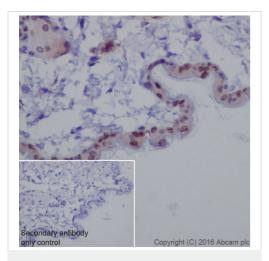
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-active YAP1 antibody [EPR19812] (ab205270)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-active YAP1 antibody
[EPR19812] (ab205270)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-active YAP1 antibody
[EPR19812] (ab205270)

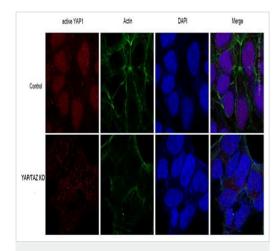
Immunohistochemical analysis of paraffin-embedded mouse skin tissue labeling active YAP1 with ab205270 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution

Mainly nuclear staining on mouse skin is observed [PMID: 21610251].

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



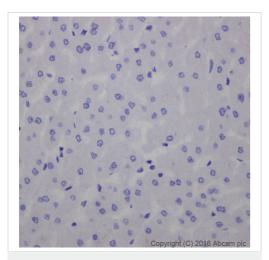
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The images showed nuclear staining on 293A cells, and background staining on YAP/TAZ knockout 293A cells.

The data was kindly provided by our collaborator Dr. Bin Zhao (Zhejiang University).

The nuclear counterstain is DAPI (blue). Counterstained with Phalloidin-technology<sup>®</sup> Alexa Fluor 488 at 1/1000 dilution.

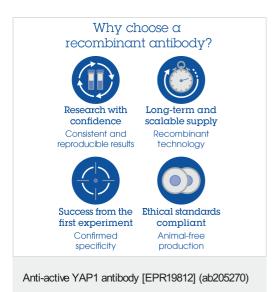


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-active YAP1 antibody
[EPR19812] (ab205270)

Immunohistochemical analysis of paraffin-embedded human liver tissue labeling active YAP1 with ab205270 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

**Negative control:** no staining on human liver [PMID:17974916]. Counter stained with Hematoxylin.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



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