abcam

Product datasheet

Anti-A-Raf antibody [EPR16208] ab200653

敲除验证 重组 RabMAb

6 References 13 图像

概述

产品名称	Anti-A-Raf抗体[EPR16208]
描述	兔单克隆抗体[EPR16208] to A-Raf
宿主	Rabbit
经测试应用	**适用于:** Flow Cyt (Intra), IHC-P, WB, ICC/IF
种属反应性	**与反应:** Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: HEK-293T, HeLa, Raji, HT-29, HEK-293, A375 and Wild-type HCT 116 whole cell lysates; Human fetal heart, fetal kidney, fetal brain and bladder lysates. IHC-P: Human cervix carcinoma and kidney tissues. ICC/IF: HeLa and HEK293 cells. Flow Cyt (intra): HeLa cells.
常规说明	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**.

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: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR16208

同种型 IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab200653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
Flow Cyt (Intra)		1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Detects a band of approximately 68 kDa (predicted molecular weight: 68 kDa).
ICC/IF		1/250.

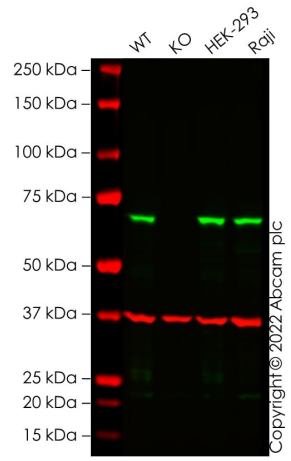
靶标

功能 Involved in the transduction of mitogenic signals from the cell membrane to the nucleus.

组织特异性 Predominantly in urogenital tissues.

序列相似性 Belongs to the protein kinase superfamily. TKL Ser/Thr protein kinase family. RAF subfamily.
Contains 1 phorbol-ester/DAG-type zinc finger.
Contains 1 protein kinase domain.
Contains 1 RBD (Ras-binding) domain.

图片



Western blot - Anti-A-Raf antibody [EPR16208] (ab200653)

All lanes : Anti-A-Raf antibody [EPR16208] (ab200653) at 1/1000 dilution

Lane 1 : Wild-type HCT 116 cell lysate
Lane 2 : A-Raf knockout HCT 116 cell lysate
Lane 3 : HEK-293 cell lysate
Lane 4 : Raji cell lysate

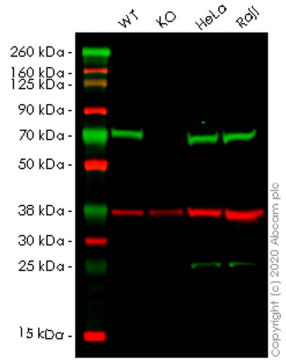
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 68 kDa
Observed band size: 67 kDa

Anti-A-Raf antibody [EPR16208] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab200653 was shown to bind specifically to A-Raf. A band was observed at 67 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in A-Raf knockout cell line **ab286752**. To generate this image, wild-type and A-Raf knockout HCT 116 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-A-Raf antibody [EPR16208] (ab200653)

All lanes : Anti-A-Raf antibody [EPR16208] (ab200653) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate
Lane 2 : A-Raf knockout HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate
Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 4 : Raji (Human Burkitts lymphoma cell line) whole cell lysate

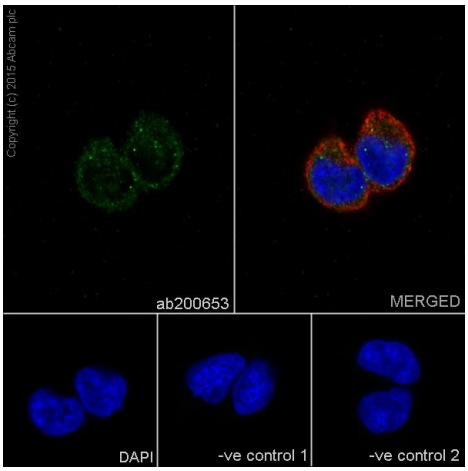
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 68 kDa
Observed band size: 68 kDa

Lanes 1-4: Merged signal (red and green). Green - ab200653 observed at 68 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab200653 Anti-A-Raf antibody [EPR16208] was shown to specifically react with A-Raf in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line **ab266351** (knockout cell lysate **ab257838**) was used. Wild-type and A-Raf knockout samples were subjected to SDS-PAGE. ab200653 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

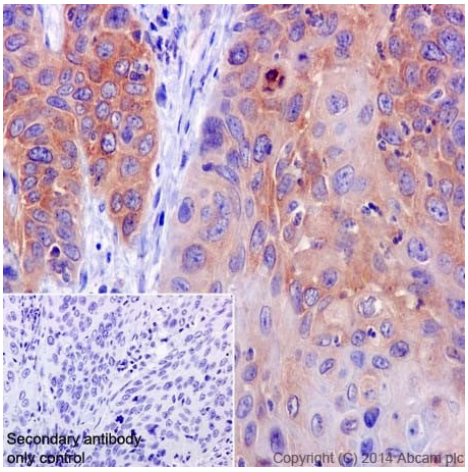


Immunocytochemistry/ Immunofluorescence - Anti-A-Raf antibody [EPR16208] (ab200653)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling A-Raf with ab200653 at 1/250 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/500 dilution (green).

Cytoplasm staining on HeLa cell line is observed.

The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).
The negative controls are as follows:
-ve control 1: ab200653 at 1/250 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2: **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-A-Raf antibody [EPR16208] (ab200653)

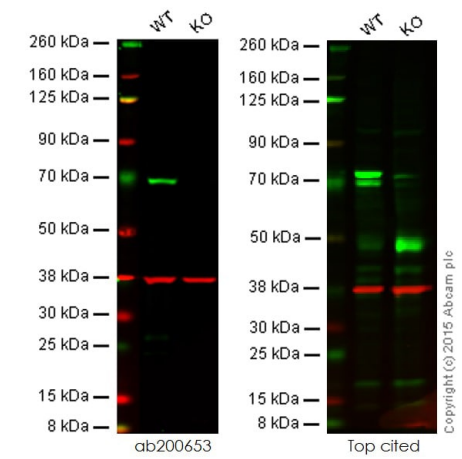
Immunohistochemical analysis of paraffin-embedded Human cervix carcinoma tissue labeling A-Raf with ab200653 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) secondary antibody at 1/500 dilution.

Cytoplasm staining on Human cervix carcinoma tissue is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG 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.

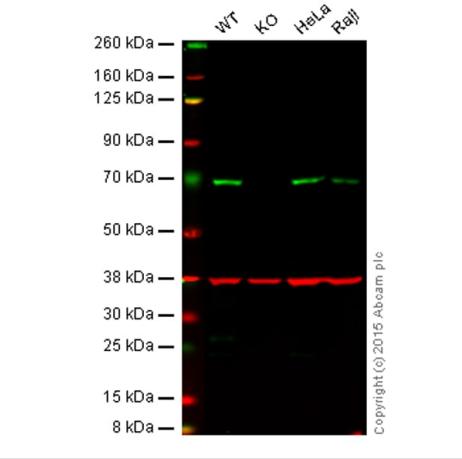


Western blot - Anti-A-Raf antibody [EPR16208] (ab200653)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: A-Raf knockout HAP1 cell lysate (20 µg)

Lanes 1 - 2: Merged signal (red and green). Green - ab200653 observed at 68 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

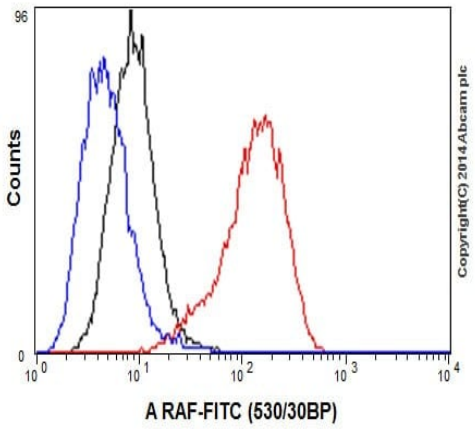
This western blot image is a comparison between ab200653 and a competitor's top cited rabbit polyclonal antibody.



Western blot - Anti-A-Raf antibody [EPR16208] (ab200653)

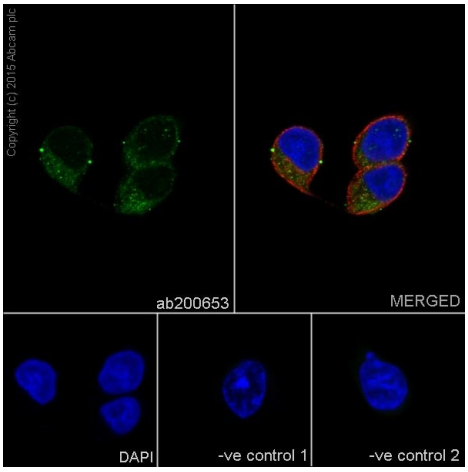
Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: A-Raf knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: Raji cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab200653 observed at 68 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab200653 was shown to specifically react with A-Raf when A-Raf knockout samples were used. Wild-type and ProteinX knockout samples were subjected to SDS-PAGE. ab200653 and **ab8245** (loading control to GAPDH) were diluted 1/1000 and 1/2000 incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and) Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) secondary antibodies at 1/10000 dilution for 1 h at room temperature before imaging.



Flow Cytometry (Intracellular) - Anti-A-Raf antibody [EPR16208] (ab200653)

Intracellular flow cytometric analysis of 2% paraformaldehyde-fixed HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling A-Raf with ab200653 at 1/100 dilution (red) compared with a rabbit monoclonal IgG isotype control (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/150 dilution was used as the secondary antibody.



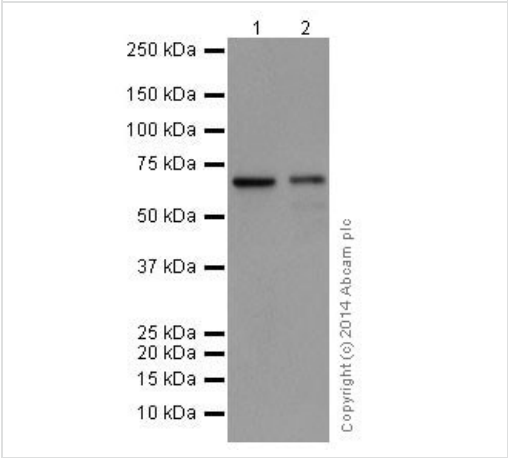
Immunocytochemistry/ Immunofluorescence - Anti-A-Raf antibody [EPR16208] (ab200653)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK293 (Human embryonic kidney) cells labeling A-Raf with ab200653 at 1/250 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/500 dilution (green).

Cytoplasm staining on HEK293 cell line is observed.

The nuclear counter stain is DAPI (blue).

Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).
The negative controls are as follows:
-ve control 1: ab200653 at 1/250 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2: **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.



Western blot - Anti-A-Raf antibody [EPR16208] (ab200653)

All lanes : Anti-A-Raf antibody [EPR16208] (ab200653) at 1/10000 dilution

Lane 1 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate
Lane 2 : Raji (Human Burkitt's lymphoma cell line) whole cell lysate

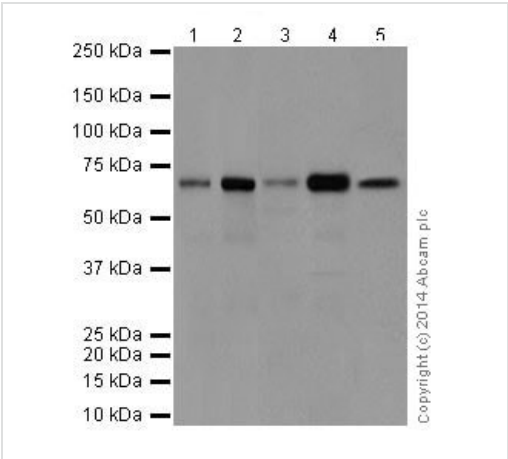
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 68 kDa
Observed band size: 68 kDa

Exposure time: 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-A-Raf antibody [EPR16208] (ab200653)

All lanes : Anti-A-Raf antibody [EPR16208] (ab200653) at 1/1000 dilution

Lane 1 : HT-29 (Human colorectal adenocarcinoma cells) whole cell lysate
Lane 2 : A375 (Human malignant melanoma) whole cell lysate
Lane 3 : Human fetal heart lysate
Lane 4 : Human fetal kidney lysate
Lane 5 : Human bladder lysate

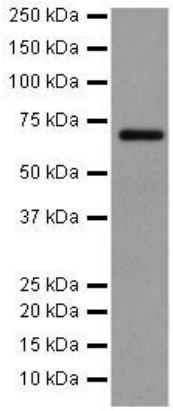
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 68 kDa
Observed band size: 68 kDa

Exposure time: 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-A-Raf antibody [EPR16208] (ab200653)

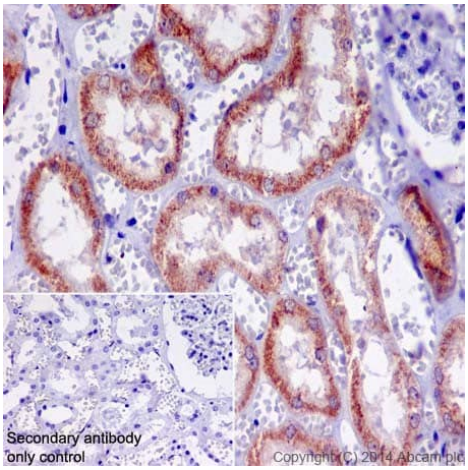
Anti-A-Raf antibody [EPR16208] (ab200653) at 1/1000 dilution + Human fetal brain lysate at 10 µg

Secondary
Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 68 kDa
Observed band size: 68 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-A-Raf antibody [EPR16208] (ab200653)

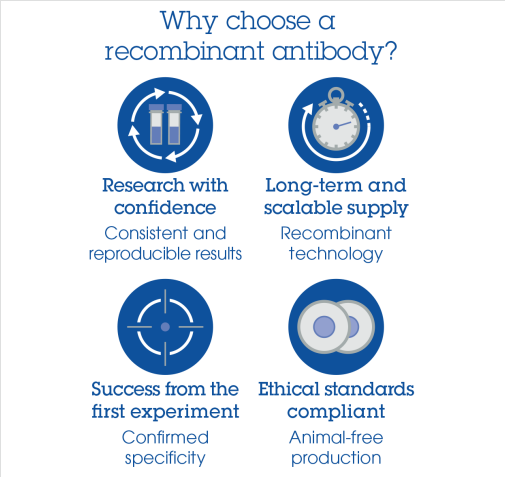
Immunohistochemical analysis of paraffin-embedded Human kidney tissue labeling A-Raf with ab200653 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) secondary antibody at 1/500 dilution.

Cytoplasm staining on Human kidney tissue is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG 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.



Anti-A-Raf antibody [EPR16208] (ab200653)

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