abcam

ab200653

Anti-A-Raf antibody [EPR16208]

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR16208
Purification technique	Affinity purification Protein A
Concentration	0.473 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/250
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/100
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/1000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Mouse
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Rat
Dilution info	-
Notes	-

Target data

See full target information ARAF

Function	Involved in the transduction of mitogenic signals from the cell membrane to the nucleus. May also regulate the TOR signaling cascade. Phosphorylates PFKFB2 (PubMed:36402789). Isoform 2. Serves as a positive regulator of myogenic differentiation by inducing cell cycle arrest, the expression of myogenin and other muscle-specific proteins, and myotube formation.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot

Storage information	Avoid freeze / thaw cycle

Notes

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

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The A-Raf protein also called ARAF is a serine/threonine-protein kinase with a molecular mass of about 68 kDa. Scientists frequently refer to it with names like A-Raf or anti A-Raf when discussing its function. A-Raf is expressed in various tissues with higher levels found in the urogenital system and the central nervous system. This protein plays an important role in cellular signal transduction processes influencing cell proliferation and differentiation through phosphorylation activity.
Biological function summary	The A-Raf protein participates in the regulation of cell growth and survival. It forms part of the MAPK/ERK signaling cascade which is essential for transmitting mitogenic signals from the cell surface to the nucleus. A-Raf acts as a modulator within this complex impacting the overall signaling output. The protein is intimately involved in the regulation of the developmental processes influencing cell fate and function.
Pathways	The A-Raf protein is central to the MAPK/ERK pathway a critical signaling pathway involved in the regulation of cellular responses to growth signals. This pathway includes key components like the proteins B-Raf and C-Raf with A-Raf playing specific roles in the modulation and fine-tuning of this signal transmission. It helps mediate signals that control cell division differentiation and secretion ensuring appropriate cellular responses to external stimuli.
Associated diseases and disorders	Aberrations in A-Raf functions have been linked to cancers particularly urogenital cancers due to its role in uncontrolled cell proliferation. Faulty A-Raf activity can also contribute to neurological disorders because of its expression in the central nervous system and involvement in neuronal signaling pathways. In these contexts it can interact with proteins like B-Raf which are important for maintaining normal cellular signaling and preventing tumorigenesis and neurodegenerative disorders.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

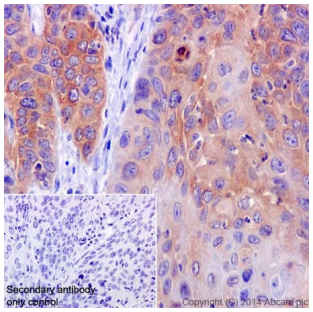
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

12 product images



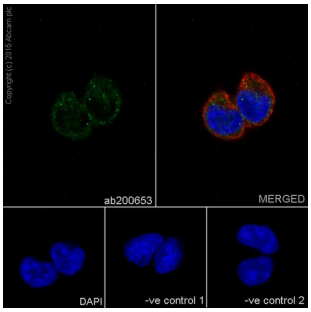
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-A-Raf antibody [EPR16208] (ab200653)

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.

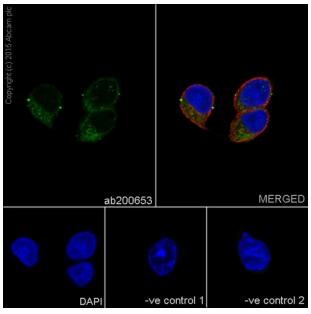


Immunocytochemistry/ Immunofluorescence - Anti-A-Raf antibody [EPR16208] (ab200653)

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.

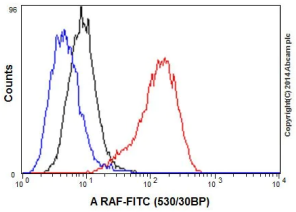


Immunocytochemistry/ Immunofluorescence - Anti-A-Raf antibody [EPR16208] (ab200653)

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.

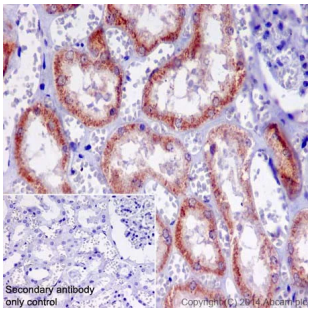
The nuclear counter stain is DAPI (blue).

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.



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.



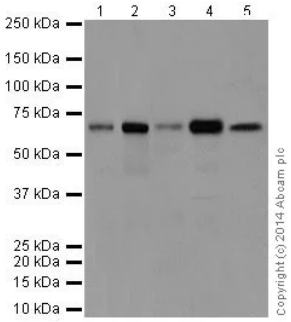
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-A-Raf antibody [EPR16208] (ab200653)

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.



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

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-A-Raf antibody [EPR16208] (ab200653) at 1/1000 dilution

Lane 1:
HT-29 (Human colorectal adenocarcinoma cells) whole cell lysate at 10 µg

Lane 2:
A375 (Human malignant melanoma) whole cell lysate at 10 µg

Lane 3:
Human fetal heart lysate at 10 µg

Lane 4:
Human fetal kidney lysate at 10 µg

Lane 5:
Human bladder lysate at 10 µg

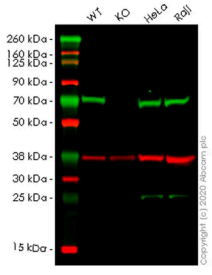
Secondary

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: 1min



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

A-Raf Western blot staining using rabbit Anti-A-Raf antibody

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

Lanes 1 - 2:
Western blot - Human A-Raf knockout HEK-293T cell lysate (ab257838) at 20 µg

Lane 1:
Wild-type HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate at 20 µg

Lane 3:
HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20 µg

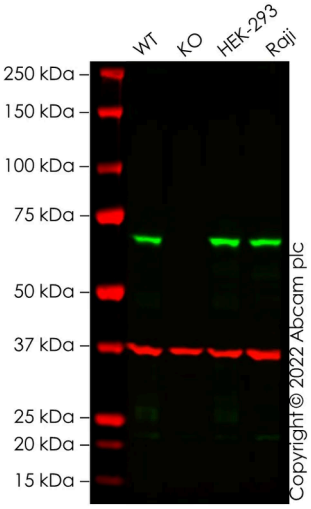
Lane 4:
Raji (Human Burkitts lymphoma cell line) whole cell lysate at 20 µg

Secondary

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

Predicted band size: 68 kDa

Observed band size: 68 kDa



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

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

Lane 1:
Wild-type HCT 116 cell lysate at 20 µg

Lane 2:
A-Raf knockout HCT 116 cell lysate at 20 µg

Lane 3:
HEK-293 cell lysate at 20 µg

Lane 4:
Raji cell lysate at 20 µg

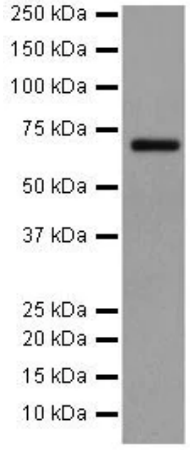
Secondary

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



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

A-Raf Western blot staining of Human fetal brain lysate using rabbit Anti-A-Raf antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-A-Raf antibody [EPR16208] (ab200653) at 1/1000 dilution

All lanes:
Human fetal brain lysate at 10 µg

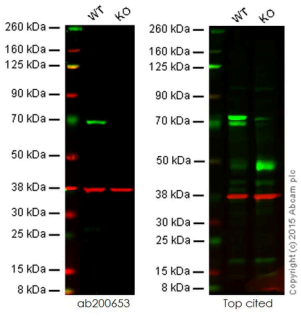
Secondary

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: 3min



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

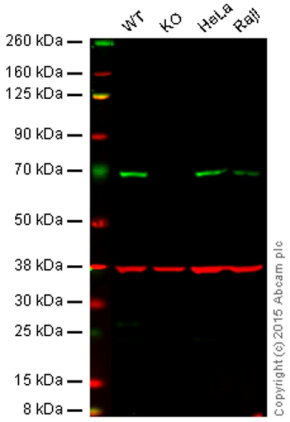
A-Raf Western blot staining using rabbit Anti-A-Raf antibody

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.

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

Predicted band size: 68 kDa

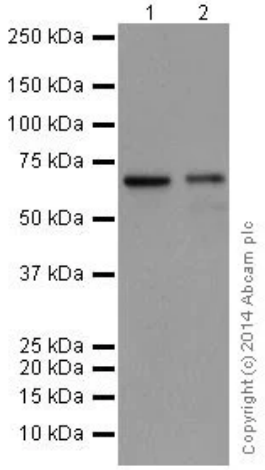


Western blot - Anti-A-Raf antibody [EPR16208] (ab200653)

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.
All lanes:
Western blot - Anti-A-Raf antibody [EPR16208] (ab200653)

Predicted band size: 68 kDa



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

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-A-Raf antibody [EPR16208] (ab200653) at 1/10000 dilution

Lane 1:
HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate at 20 µg

Lane 2:
Raji (Human Burkitt's lymphoma cell line) whole cell lysate at 20 µg

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: 1min

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.