

## Product datasheet

# Anti-A RAF antibody [EPR16208] ab200653

**KO VALIDATED** Recombinant RabMAb<sup>®</sup>

1 References 10 Images

### Overview

<b>Product name</b>	Anti-A RAF antibody [EPR16208]
<b>Description</b>	Rabbit monoclonal [EPR16208] to A RAF
<b>Host species</b>	Rabbit
<b>Tested applications</b>	<b>Suitable for:</b> IHC-P, WB, ICC/IF, Flow Cyt
<b>Species reactivity</b>	<b>Reacts with:</b> Human
<b>Immunogen</b>	Recombinant fragment within Human A RAF aa 150-300. The exact sequence is proprietary. Database link: <a href="#">P10398</a>
<b>Positive control</b>	WB: HeLa, Raji, HT-29 and A375 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: HeLa cells.
<b>General notes</b>	

Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to [RabMAb<sup>®</sup> patents](#).

This product is a [recombinant rabbit monoclonal antibody](#).

### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	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.
<b>Storage buffer</b>	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EPR16208
<b>Isotype</b>	IgG

## Applications

Our [Abpromise guarantee](#) covers the use of **ab200653** 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
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.
Flow Cyt		1/100. <a href="#">ab172730</a> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

## Target

### Function

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

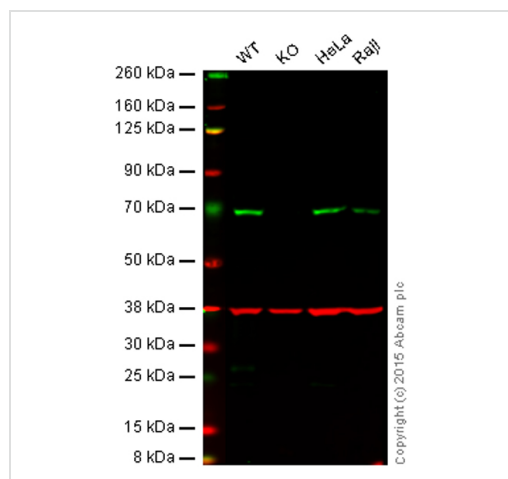
### Tissue specificity

Predominantly in urogenital tissues.

### Sequence similarities

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.

## Images



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

**Lane 1:** Wild-type HAP1 cell lysate (20 µg)

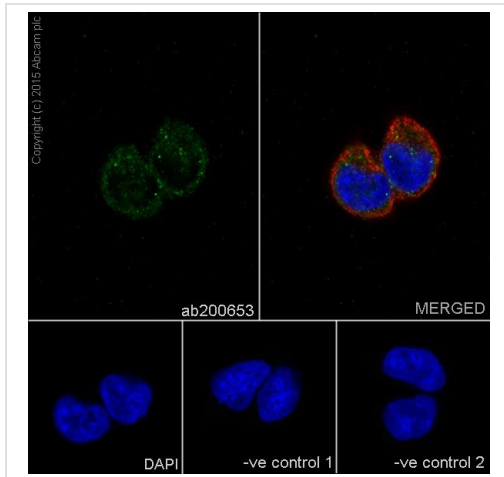
**Lane 2:** A RAF knockout HAP1 cell lysate (20 µg)

**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.



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).

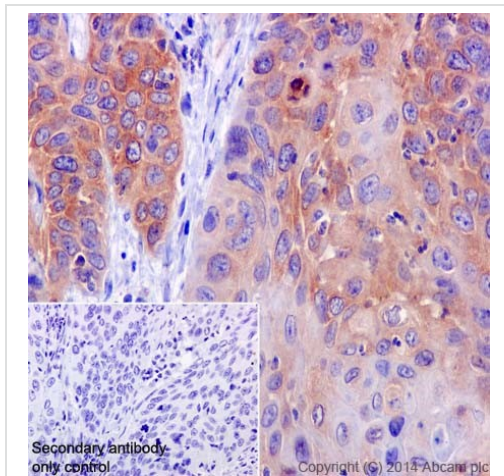
Cytoplasm staining on HeLa cell line is observed.

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)

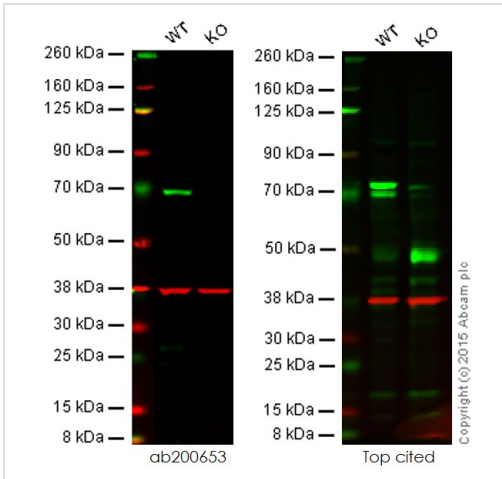
Immunohistochemical analysis of paraffin-embedded Human cervix carcinoma tissue labeling A RAF with ab200653 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution.

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.



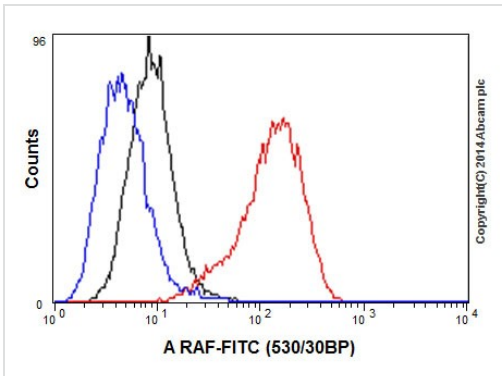
Western blot - Anti-A RAF antibody [EPR16208]  
(ab200653)

**Lane 1:** Wild-type HAP1 cell lysate (20 µg)

**Lane 2:** A RAF knockout HAP1 cell lysate (20 µg)

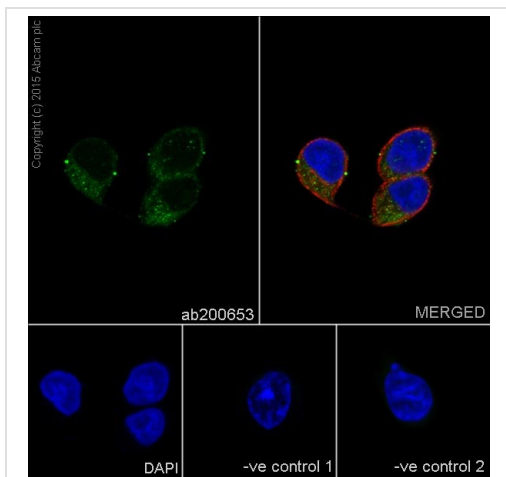
**Lanes 1 - 2:** Merged signal (red and green). Green - ab200653 observed at 68 kDa. Red - loading control, ab8245, observed at 37 kDa.

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



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

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.

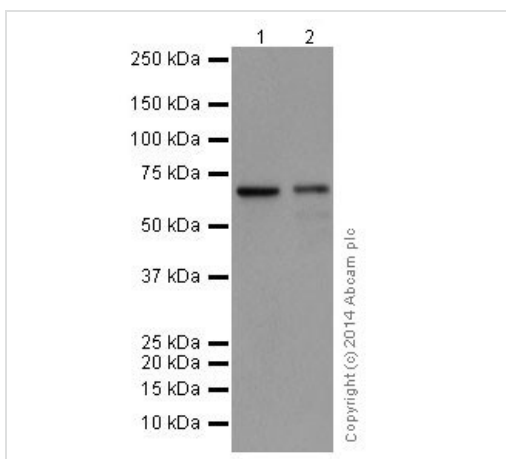
The nuclear counter stain is DAPI (blue).

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

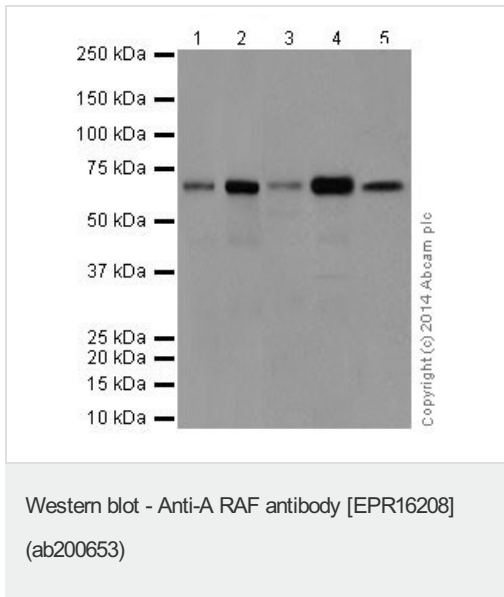
**All lanes** : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

**Predicted band size:** 68 kDa

**Observed band size:** 68 kDa

**Exposure time:** 1 minute

Blocking/Dilution buffer: 5% NFDm/TBST.



**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**

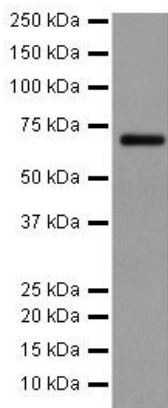
**All lanes :** Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

**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**

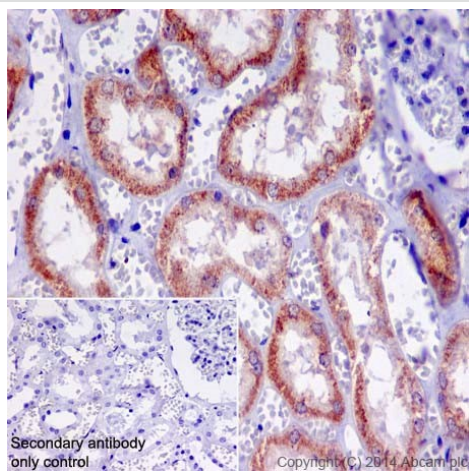
Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

**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.

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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