

# Anti-A-Raf antibody [EPR16208] - BSA and Azide free ab240354

KO VALIDATED Recombinant RabMAb

8 Images

### Overview

Product name	Anti-A-Raf antibody [EPR16208] - BSA and Azide free
Description	Rabbit monoclonal [EPR16208] to A-Raf - BSA and Azide free
Host species	Rabbit
Tested applications	<b>Suitable for:</b> WB, Flow Cyt (Intra), ICC/IF, IHC-P
Species reactivity	<b>Reacts with:</b> Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	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.
General notes	<p>ab240354 is the carrier-free version of <a href="#">ab200653</a>.</p> <p>Our <b>carrier-free</b> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.</p> <p>This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.</p> <p>Use our <b>conjugation kits</b> for antibody conjugates that are ready-to-use in as little as 20 minutes with &lt;1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.</p> <p>This product is compatible with the Maxpar<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> is a trademark of Fluidigm Canada Inc.</p> <p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit</p>

monoclonal antibodies. For details on our patents, please refer to [\*\*RabMAb® patents\*\*](#).

## Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR16208
Isotype	IgG

## Applications

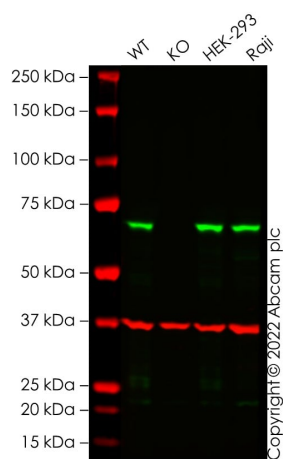
**The Abpromise guarantee** Our [\*\*Abpromise guarantee\*\*](#) covers the use of ab240354 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 68 kDa (predicted molecular weight: 68 kDa).
Flow Cyt (Intra)		Use at an assay dependent concentration. <b>ab199376</b> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

## 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] - BSA and Azide free (ab240354)

**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

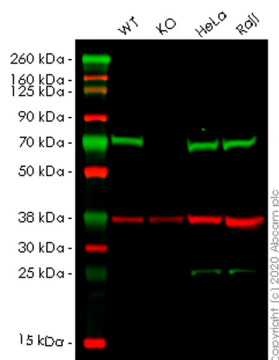
Performed under reducing conditions.

**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.

This data was developed using [ab200653](#), the same antibody clone in a different buffer formulation.



Western blot - Anti-A-Raf antibody [EPR16208] - BSA and Azide free (ab240354)

**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

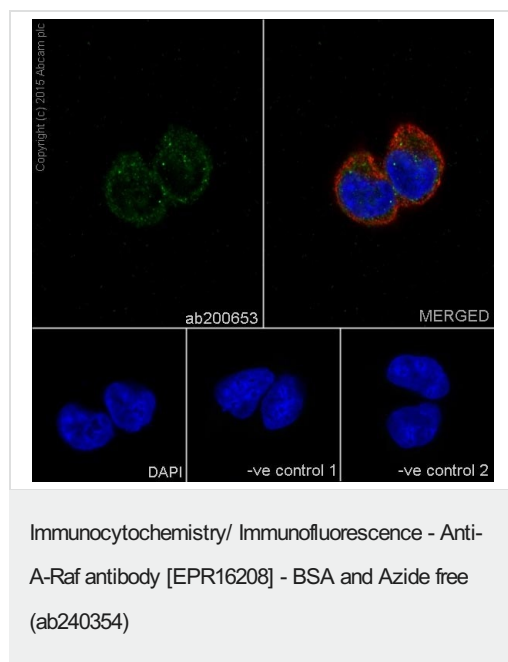
**Predicted band size:** 68 kDa

**Observed band size:** 68 kDa

This data was developed using [ab200653](#), the same antibody clone in a different buffer formulation.

**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.



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.

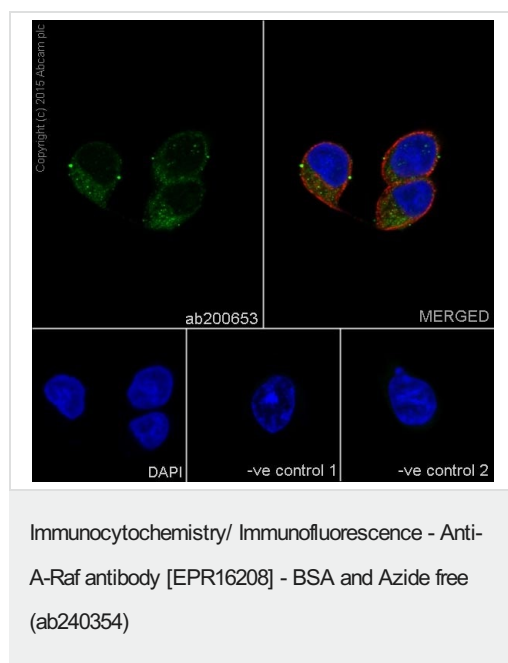
The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**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).

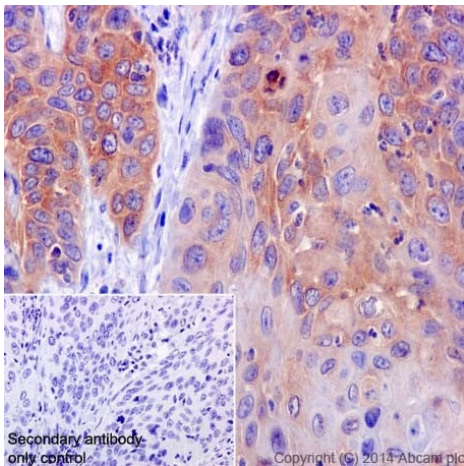
Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab200653**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-A-Raf antibody [EPR16208] - BSA and Azide free (ab240354)

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.

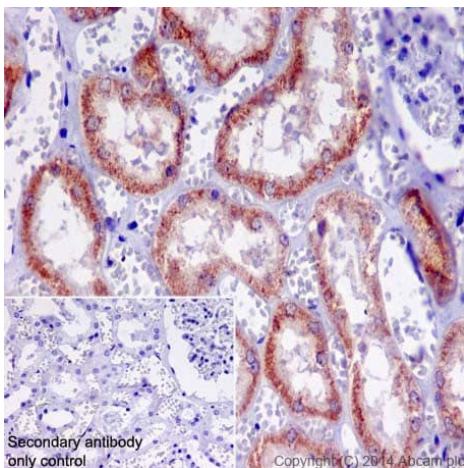
Cytoplasm staining on Human cervix carcinoma tissue is observed.

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab200653**).

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



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-A-Raf antibody [EPR16208] - BSA and Azide free (ab240354)

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.

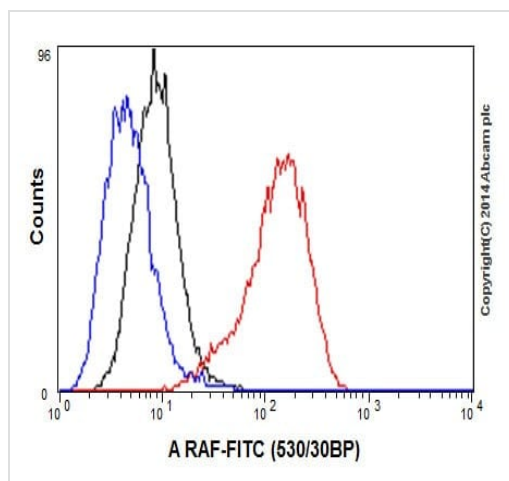
Cytoplasm staining on Human kidney tissue is observed.

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab200653**).

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



Flow Cytometry (Intracellular) - Anti-A-Raf antibody  
[EPR16208] - BSA and Azide free (ab240354)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab200653**).

### Why choose a recombinant antibody?



**Research with confidence**  
Consistent and reproducible results



**Long-term and scalable supply**  
Recombinant technology



**Success from the first experiment**  
Confirmed specificity



**Ethical standards compliant**  
Animal-free production

Anti-A-Raf antibody [EPR16208] - BSA and Azide free (ab240354)

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

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