abcam

Product datasheet

Anti-BRAF antibody [EP152Y] - BSA and Azide free ab189351

KO VALIDATED Recombinant RabMAb

5 References 7 Images

Overview

Product name	Anti-BRAF antibody [EP152Y] - BSA and Azide free
Description	Rabbit monoclonal [EP152Y] to BRAF - BSA and Azide free
Host species	Rabbit
Specificity	The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.
Tested applications	**Suitable for:** WB, IHC-P, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa (**ab150035**) and HAP1 whole cell lysate. IHC-P: human prostate cancer tissue. This antibody also reacts with rat brain tissue. IP: HeLa cells.
General notes	ab189351 is the carrier-free version of **ab33899**. Our **carrier-free** 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. 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. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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**.

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	EP152Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab189351 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 87 kDa (predicted molecular weight: 85 kDa).
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. See **IHC antigen retrieval protocols**. The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.
IP		Use at an assay dependent concentration.

Target

Function	Involved in the transduction of mitogenic signals from the cell membrane to the nucleus. May play a role in the postsynaptic responses of hippocampal neuron.
Tissue specificity	Brain and testis.
Involvement in disease	Note=Defects in BRAF are found in a wide range of cancers. Defects in BRAF may be a cause of colorectal cancer (CRC) [MIM:114500]. Defects in BRAF are involved in lung cancer (LNCR) [MIM:211980]. Defects in BRAF are involved in non-Hodgkin lymphoma (NHL) [MIM:605027]. NHL is a cancer that starts in cells of the lymph system, which is part of the body's immune system. NHLs can

occur at any age and are often marked by enlarged lymph nodes, fever and weight loss.
Defects in BRAF are a cause of cardiofaciocutaneous syndrome (CFC syndrome) [MIM:115150]; also known as cardio-facio-cutaneous syndrome. CFC syndrome is characterized by a distinctive facial appearance, heart defects and mental retardation. Heart defects include pulmonic stenosis, atrial septal defects and hypertrophic cardiomyopathy. Some affected individuals present with ectodermal abnormalities such as sparse, friable hair, hyperkeratotic skin lesions and a generalized ichthyosis-like condition. Typical facial features are similar to Noonan syndrome. They include high forehead with bitemporal constriction, hypoplastic supraorbital ridges, downslanting palpebral fissures, a depressed nasal bridge, and posteriorly angulated ears with prominent helices. The inheritance of CFC syndrome is autosomal dominant.
Defects in BRAF are the cause of Noonan syndrome type 7 (NS7) [MIM:613706]. Noonan syndrome is a disorder characterized by facial dysmorphic features such as hypertelorism, a downward eyeslant and low-set posteriorly rotated ears. Other features can include short stature, a short neck with webbing or redundancy of skin, cardiac anomalies, deafness, motor delay and variable intellectual deficits.
Defects in BRAF are the cause of LEOPARD syndrome type 3 (LEOPARD3) [MIM:613707]. LEOPARD3 is a disorder characterized by lentigines, electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonic stenosis, abnormalities of genitalia, retardation of growth, and sensorineural deafness.
Note=A chromosomal aberration involving BRAF is found in pilocytic astrocytomas. A tandem duplication of 2 Mb at 7q34 leads to the expression of a KIAA1549-BRAF fusion protein with a constitutive kinase activity and inducing cell transformation.

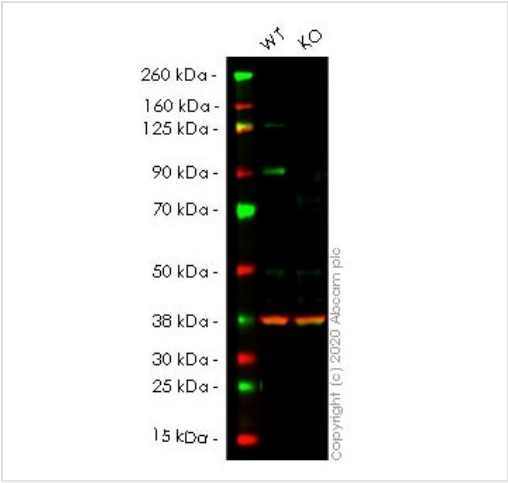
Sequence similarities

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.

Cellular localization

Nucleus. Cytoplasm. Cell membrane. Colocalizes with RGS14 and RAF1 in both the cytoplasm and membranes.

Images



Western blot - Anti-BRAF antibody [EP152Y] - BSA and Azide free (ab189351)

All lanes : Anti-BRAF antibody [EP152Y] (**ab33899**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : BRAF knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

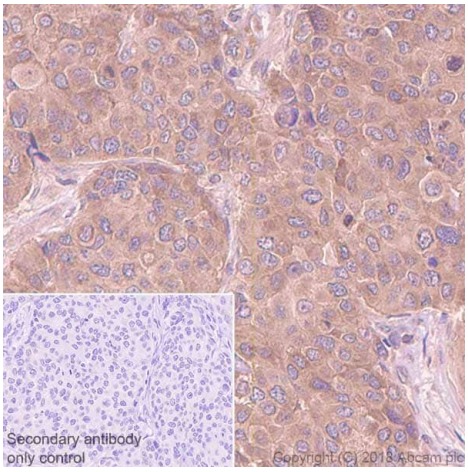
Predicted band size: 85 kDa
Observed band size: 90 kDa

This data was developed using the same antibody clone in a

different buffer formulation (**ab33899**).

Lanes 1- 2: Merged signal (red and green). Green - **ab33899** observed at 90 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

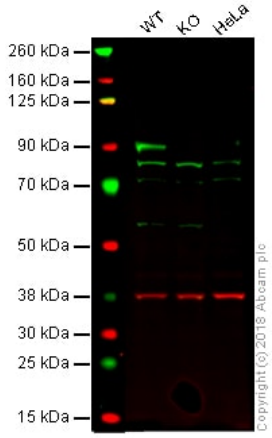
ab33899 was shown to react with BRAF in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab265373** (knockout cell lysate **ab257078**) was used. Wild-type HeLa and BRAF knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab33899** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 dilution and a 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BRAF antibody [EP152Y] - BSA and Azide free (ab189351)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human breast cancer tissue sections labeling BRAF with purified **ab33899** at 1/100 dilution (4.77 µg/ml). Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab33899**).



Western blot - Anti-BRAF antibody [EP152Y] - BSA and Azide free (ab189351)

All lanes : Anti-BRAF antibody [EP152Y] (**ab33899**) at 1/1000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : BRAF knockout HAP1 whole cell lysate
Lane 3 : HeLa whole cell lysate

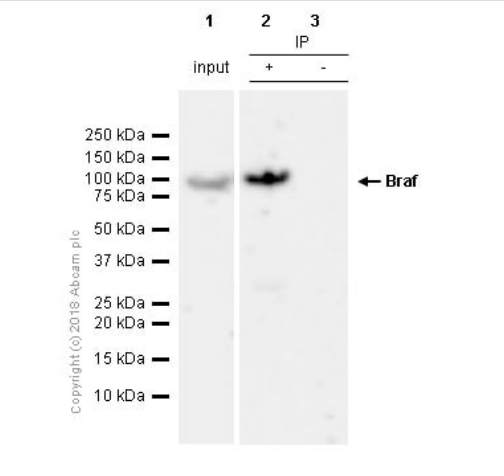
Lysates/proteins at 40 µg per lane.

Predicted band size: 85 kDa

Lanes 1 - 3: Merged signal (red and green). Green - **ab33899** (unpurified) observed at 90 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

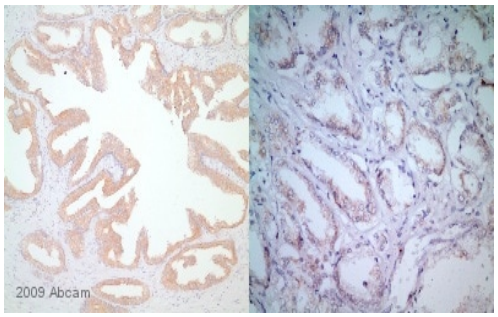
ab33899 was shown to recognize BRAF in wild-type HAP1 cells as signal was lost at the expected MW in BRAF knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and BRAF knockout samples were subjected to SDS-PAGE. **ab33899** and **ab8245** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab33899**).



Immunoprecipitation - Anti-BRAF antibody [EP152Y] - BSA and Azide free (ab189351)

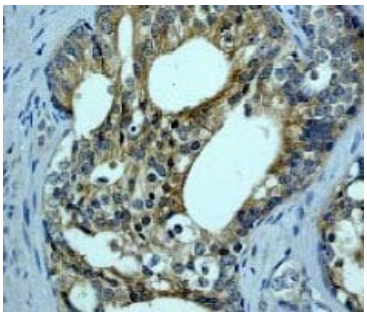
ab33899 (Purified) at 1:500 dilution (0.954 µg/ml) immunoprecipitating BRAF in HeLa whole cell lysate .
Lane 1 (input): HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg
Lane 2 (+): **ab33899** & HeLa whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab33899** in HeLa whole cell lysate
For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1:1000 dilution.
Blocking and diluting buffer: 5% NFDM /TBST .This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab189351)



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BRAF antibody [EP152Y] - BSA and Azide free (ab189351)

This IHC data was generated using the same anti-B Raf antibody clone, EP152Y, in a different buffer formulation (cat# **ab33899**).

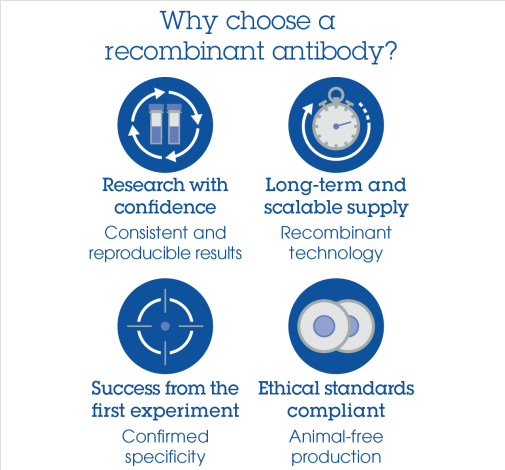
ab33899 (unpurified) staining B Raf cells from human prostate tissue by immunohistochemistry (formalin/PFA-fixed paraffin-embedded sections). Cells were formaldehyde fixed and permeabilized in PBS-Tween 20 prior to blocking in 70% serum for 10 minutes at 25°C. The primary antibody was diluted 1/250 and incubated with the sample for 1 hour at 25°C. A biotin conjugated goat polyclonal to mouse Ig was used as the secondary.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BRAF antibody [EP152Y] - BSA and Azide free (ab189351)

This IHC data was generated using the same anti-B Raf antibody clone, EP152Y, in a different buffer formulation (cat# **ab33899**).

This image shows paraffin embedded human prostate cancer tissue sample stained with **ab33899** (unpurified) at 1/250 dilution.



Anti-BRAF antibody [EP152Y] - BSA and Azide free

(ab189351)

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

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors