abcam

Product datasheet

Anti-Raf1 antibody [EP4969] - BSA and Azide free ab236003

KO VALIDATED Recombinant RabMAb

1 References 7 Images

Overview

Product name	Anti-Raf1 antibody [EP4969] - BSA and Azide free
Description	Rabbit monoclonal [EP4969] to Raf1 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1, HeLa, K-562, RAW 264.7, C2C12, NIH/3T3, and PC-12 cell lysates. ICC/IF: HeLa and K562 cells. Flow Cyt (intra): K562 cells.
General notes	ab236003 is the carrier-free version of **ab181115**. 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	EP4969
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab236003 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

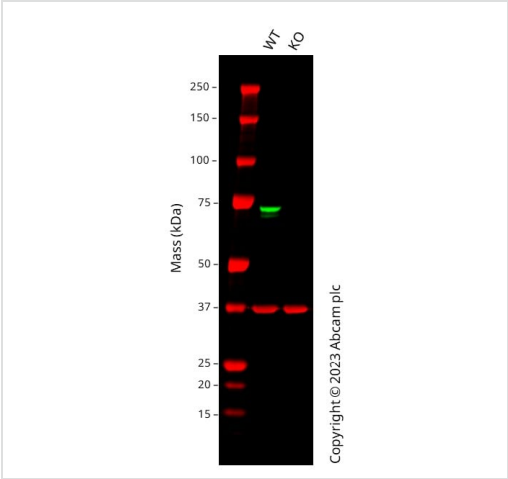
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 73 kDa (predicted molecular weight: 73 kDa).

Target

Function	Involved in the transduction of mitogenic signals from the cell membrane to the nucleus. Part of the Ras-dependent signaling pathway from receptors to the nucleus. Protects cells from apoptosis mediated by STK3.
Tissue specificity	In skeletal muscle, isoform 1 is more abundant than isoform 2.
Involvement in disease	Defects in RAF1 are the cause of Noonan syndrome type 5 (NS5) [MIM:611553]. Noonan syndrome (NS) is a disorder characterized by dysmorphic facial features, short stature, hypertelorism, cardiac anomalies, deafness, motor delay, and a bleeding diathesis. It is a genetically heterogeneous and relatively common syndrome, with an estimated incidence of 1 in 1000-2500 live births. Defects in RAF1 are the cause of LEOPARD syndrome type 2 (LEOPARD2) [MIM:611554]. LEOPARD syndrome is an autosomal dominant disorder allelic with Noonan syndrome. The acronym LEOPARD stands for lentigines, electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonic stenosis, abnormalities of genitalia, retardation of growth, and deafness.
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.

Post-translational modifications	Phosphorylated upon DNA damage, probably by ATM or ATR. Phosphorylation at Thr-269 increases its kinase activity. Phosphorylation at Ser-259 induces the interaction with YWHAZ and inactivates kinase activity. Dephosphorylation of Ser-259 by the complex containing protein phosphatase 1, SHOC2 and M-Ras/MRAS relieves inactivation, leading to stimulate RAF1 activity.
Cellular localization	Cytoplasm. Cell membrane. Colocalizes with RGS14 and BRAF in both the cytoplasm and membranes.

Images



Western blot - Anti-Raf1 antibody [EP4969] - BSA and Azide free (ab236003)

All lanes : Anti-Raf1 antibody [EP4969] - N-terminal (**ab181115**) at 1/1000 dilution

Lane 1 : Wild-type HCT 116 cell lysate
Lane 2 : RAF1 knockout HCT 116 cell lysate

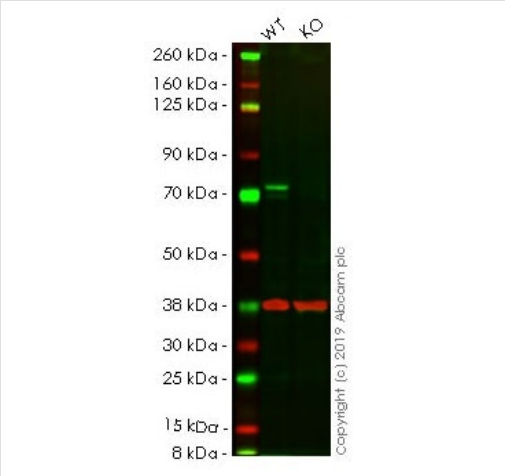
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 73 kDa
Observed band size: 73 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab181115**).

Western blot: Anti-RAF1 antibody [EP4969] (**ab181115**) 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, **ab181115** was shown to bind specifically to RAF1. A band was observed at 73 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in RAF1 knockout cell line. To generate this image, wild-type and RAF1 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-Raf1 antibody [EP4969] - BSA and Azide free (ab236003)

All lanes : Anti-Raf1 antibody [EP4969] - N-terminal (**ab181115**) at 1/20000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : RAF1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

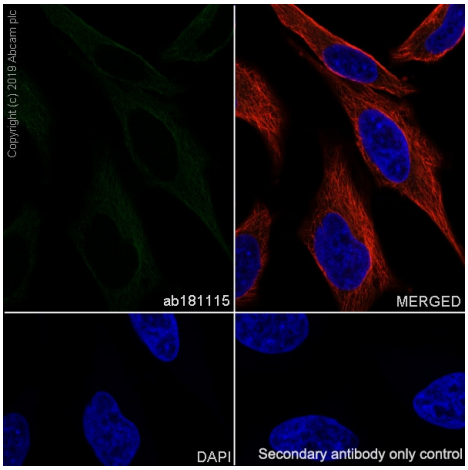
Performed under reducing conditions.

Predicted band size: 73 kDa
Observed band size: 73 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab181115**).

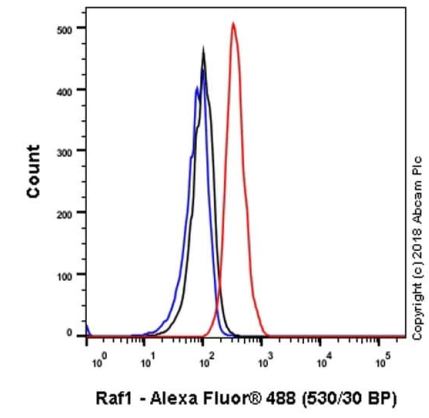
Lanes 1- 2: Merged signal (red and green). Green - **ab181115** observed at 73 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab181115 was shown to react with Raf1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab264978** (knockout cell lysate **ab257126**) was used. Wild-type HeLa and RAF1 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. **ab181115** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 20000 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.



Immunocytochemistry/ Immunofluorescence - Anti-Raf1 antibody [EP4969] - BSA and Azide free (ab236003)

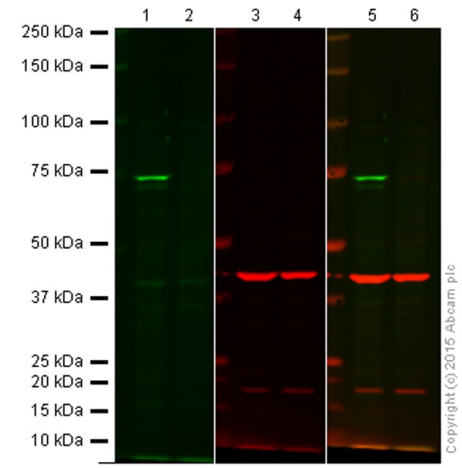
Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Raf1 with purified **ab181115** at 1/100 dilution (10 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab181115**)



Flow Cytometry (Intracellular) - Anti-Raf1 antibody [EP4969] - BSA and Azide free (ab236003)

Intracellular Flow Cytometry analysis of K-562 (Human chronic myelogenous leukemia lymphoblast) cells labelling with **ab181115** at 1/100 dilution (10.79µg/mL) (red). Cells were fixed with 4% paraformaldehyde . Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/2000 dilution. Isotype control - 90% methanol . Unlabeled control - Rabbit monoclonal IgG (**ab172730**) / Black.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab181115**).



Western blot - Anti-Raf1 antibody [EP4969] - BSA and Azide free (ab236003)

Lanes 1-2 : Anti-Raf1 antibody [EP4969] - N-terminal (**ab181115**) at 1/1000 dilution
Lanes 3-4 : Anti-beta Actin antibody [mAbcam 8226] - Loading Control (**ab8226**) at 1/1000 dilution

Lanes 1 & 3 & 5 : Wild-type HAP1 cell lysate
Lanes 2 & 4 & 6 : Raf1 knockout HAP1 cell lysate

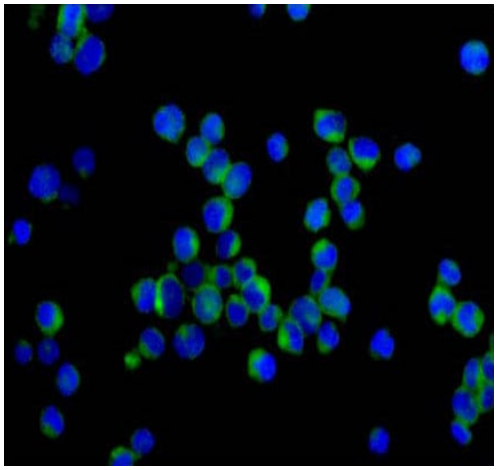
Lysates/proteins at 20 µg per lane.

Predicted band size: 73 kDa

Lanes 1 and 2: Green signal from target – **ab181115** observed at 74 kDa
Lanes 3 and 4: Red signal from loading control – **ab8226** observed at 42 kDa
Lanes 5 and 6: Merged (red and green) signal

ab181115 was shown to specifically react with Raf1 when Raf1 knockout samples were used. Wild-type and Raf1 knockout samples were subjected to SDS-PAGE. **ab181115** and **ab8226** (loading control to beta actin) were both diluted 1/1000 and incubated overnight at 4ºC. 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 1/10000 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 (**ab181115**).



Immunocytochemistry/ Immunofluorescence - Anti-Raf1 antibody [EP4969] - BSA and Azide free (ab236003)

Immunofluorescent analysis of acetone-fixed K562 cells labeling Raf1 with **ab181115** at 1/250 dilution, followed by Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody at 1/200 dilution. Counter stained with Dapi.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab181115**).

Anti-Raf1 antibody [EP4969] - BSA and Azide free (ab236003)

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