abcam

Product datasheet

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

KO VALIDATED | Recombinant | RabMAb

4 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:** ICC/IF, Flow Cyt, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human Raf1 aa 1-100. The exact sequence is proprietary. Database link: P04049
Positive control	WB: HeLa, K-562, RAW 264.7, C2C12, NIH/3T3, and PC-12 lysates. ICC/IF: HeLa cells.
General notes	Ab236003 is the carrier-free version of ab181115. This format is designed for use in antibody labeling, including fluorochromes, metal isotopes, oligonucleotides, enzymes. Our carrier-free formats are supplied in a buffer free of BSA, sodium azide and glycerol for higher conjugation efficiency. 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. ab236003 is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm. *Maxpar® is a trademark of Fluidigm Canada Inc.* Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents. **We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.** This product is a recombinant rabbit monoclonal antibody.

Properties

Properties

Form	Liquid
Storage instructions	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.
Storage buffer	Constituent: PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP4969
Isotype	IgG

Applications

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.

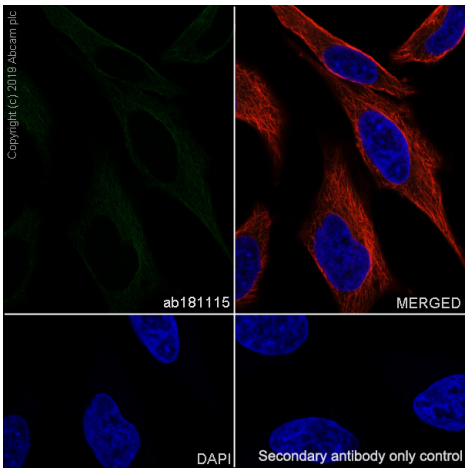
Application	Abreviews	Notes
ICC/IF		Use at an assay dependent concentration.
Flow Cyt		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 73 kDa (predicted molecular weight: 73 kDa).

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

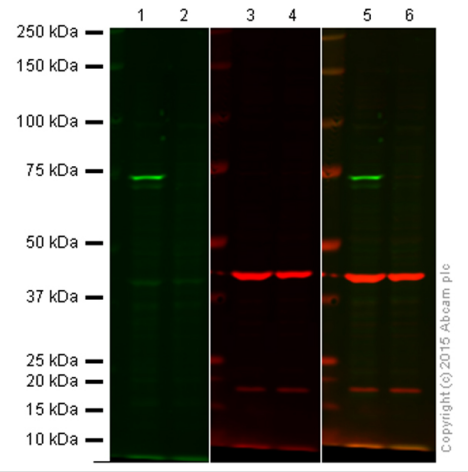
	phosphatase 1, SHOC2 and M-Ras/MRAS relieves inactivation, leading to stimulate RAF1 activity.
Cellular localization	Cytoplasm. Cell membrane. Colocalizes with RGS14 and BRAF in both the cytoplasm and membranes.

Images



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)

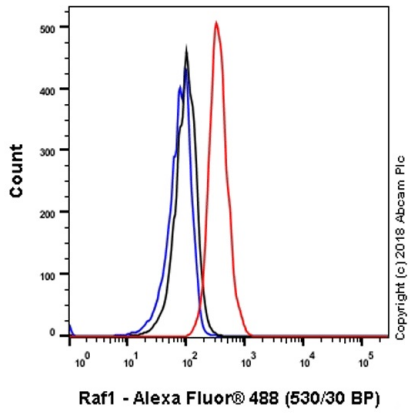


Western blot - Anti-Raf1 antibody [EP4969] - BSA and Azide free (ab236003)

Lanes 1, 3 and 5: Wild-type HAP1 cell lysate (20 µg)
Lanes 2, 4 and 6: Raf1 knockout HAP1 cell lysate (20 µg)
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.

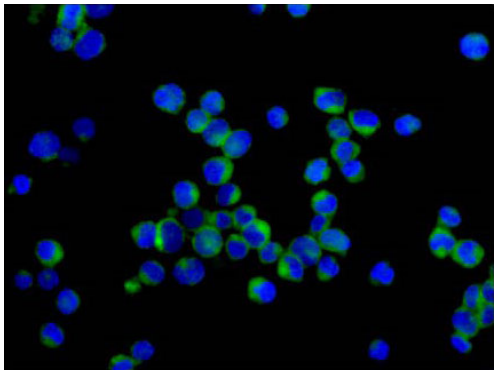
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab181115).



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

Flow cytometry analysis of K-562 (Human chronic myelogenous leukemia lymphoblast) cells labelling with ab181115 at 1/100 dilution (10.79 µg/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).



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).

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