abcam

Product datasheet

Anti-Raf1 (phospho S259) antibody [EPR3433(2)] ab173539

Recombinant RabMAb

13 References 7 Images

Overview

Product name	Anti-Raf1 (phospho S259) antibody [EPR3433(2)]
Description	Rabbit monoclonal [EPR3433(2)] to Raf1 (phospho S259)
Host species	Rabbit
Tested applications	**Suitable for:** WB, Dot blot **Unsuitable for:** Flow Cyt,ICC/IF or IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Treated C6, NIH/3T3 and HEK-293 whole cell lysates.
General notes	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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3433(2)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab173539 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		1/1000 - 1/5000. Predicted molecular weight: 73 kDa.
Dot blot		Use at an assay dependent concentration.

Application notes Is unsuitable for Flow Cyt,ICC/IF or IP.

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.

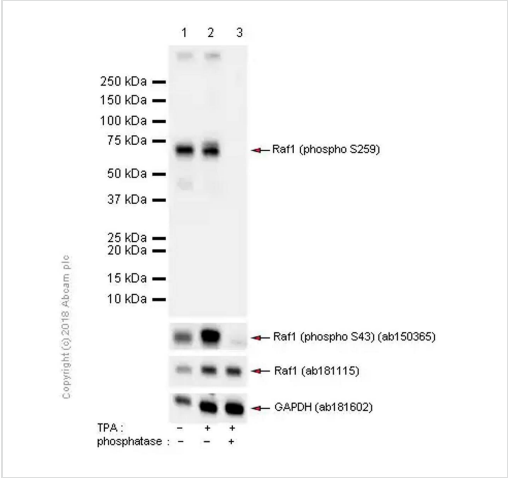
Involvement in disease Defects in RAF1 are the cause of Noonan syndrome type 5 (NS5) [MIM:611553]. Noonan syndrome (NS) is a disorder characterized by dysmorphic facial features, short stature, hypertelorism, cardiac anomalies, deafness, motor delay, and a bleeding diathesis. It is a genetically heterogeneous 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 (phospho S259) antibody [EPR3433(2)] (ab173539)

All lanes : Anti-Raf1 (phospho S259) antibody [EPR3433(2)] (ab173539) at 1/5000 dilution (Purified)

Lane 1 : HEK-293 (Human embryonic kidney epithelial cell) whole cell lysates

Lane 2 : HEK-293 (Human embryonic kidney epithelial cell) treated with 100nM Phorbol-12-myristate-13-acetate for 1 hour whole cell lysates

Lane 3 : HEK-293 (Human embryonic kidney epithelial cell) treated with 100nM Phorbol-12-myristate-13-acetate for 1 hour whole cell lysates, then incubated with phosphatase

Lysates/proteins at 15 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

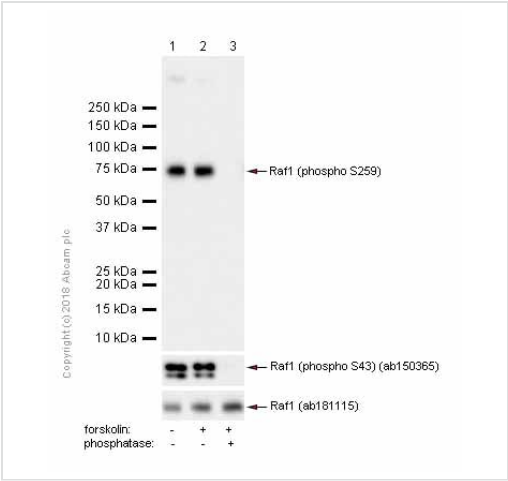
Predicted band size: 73 kDa

Observed band size: 73 kDa

ab181602 was used as GAPDH loading control.

ab181115 was used to detect Raf1.

ab150365 was used to detect Raf1 phospho S43.



Western blot - Anti-Raf1 (phospho S259) antibody [EPR3433(2)] (ab173539)

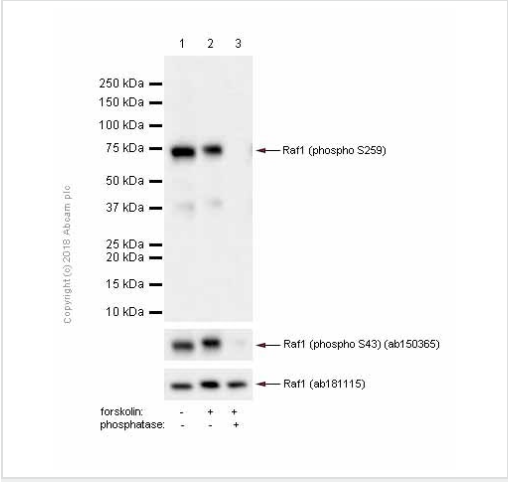
All lanes : Anti-Raf1 (phospho S259) antibody [EPR3433(2)] (ab173539) at 1/1000 dilution (Purified)

Lane 1 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) treated with 5uM forskolin for 10 minutes whole cell lysates
Lane 3 : NIH/3T3 (Mouse embryonic fibroblast) treated with 5uM forskolin for 10 minutes whole cell lysates, then incubated with phosphatase

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

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



Western blot - Anti-Raf1 (phospho S259) antibody [EPR3433(2)] (ab173539)

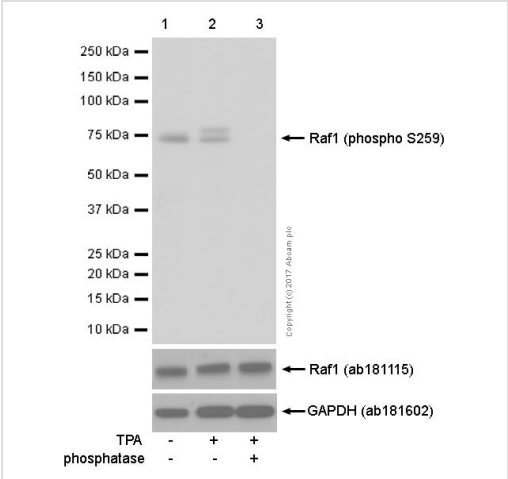
Lane 1 : Anti-Raf1 (phospho S259) antibody [EPR3433(2)] (ab173539) at 1/1000 dilution (Purified)
Lanes 2-3 : Anti-Raf1 (phospho S259) antibody [EPR3433(2)] (ab173539) at 1/1000 dilution

Lane 1 : C6 (Rat glial tumor glial cell) whole cell lysates
Lane 2 : C6 (Rat glial tumor glial cell) treated with 30uM forskolin for 20 minutes whole cell lysates
Lane 3 : C6 (Rat glial tumor glial cell) treated with 30uM forskolin for 20 minutes whole cell lysates, then incubated with phosphatase

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

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



Western blot - Anti-Raf1 (phospho S259) antibody [EPR3433(2)] (ab173539)

All lanes : Anti-Raf1 (phospho S259) antibody [EPR3433(2)] (ab173539) at 1/5000 dilution

Lane 1 : HEK293 whole cell lysate
Lane 2 : HEK293 whole cell lysate treated with 100nM Phorbol-12-myristate-13-acetate for 1 hour
Lane 3 : HEK293 whole cell lysate treated with 100nM Phorbol-12-myristate-13-acetate for 1 hour then incubated with phosphatase

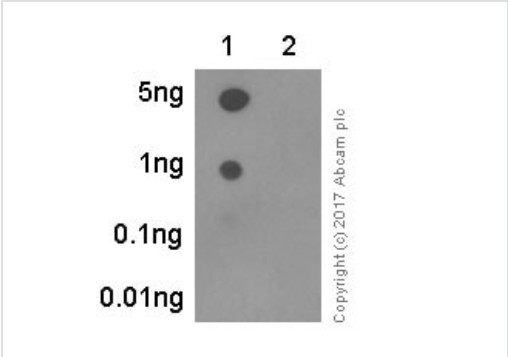
Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

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

Exposure time: 1 second

Blocking and dilution buffer: 5% NFDM/TBST.

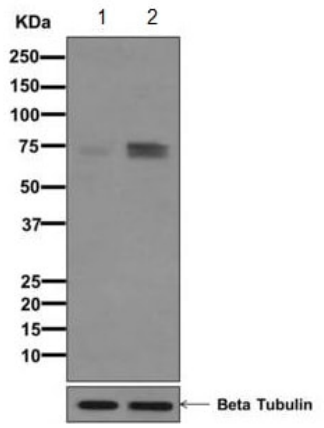


Dot Blot - Anti-Raf1 (phospho S259) antibody [EPR3433(2)] (ab173539)

Dot blot analysis of Raf1 (pS259) peptide (Lane 1) and Raf1 non-phospho peptide (Lane 2) labelling Raf1 (pS259) with ab173539 at a dilution of 1/1000. **ab97051** (Peroxidase conjugated goat anti-rabbit IgG (H+L)) was used as the secondary antibody at a dilution of 1/100000.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



Western blot - Anti-Raf1 (phospho S259) antibody [EPR3433(2)] (ab173539)

All lanes : Anti-Raf1 (phospho S259) antibody [EPR3433(2)] (ab173539) at 1/1000 dilution

Lane 1 : 293T cell lysates

Lane 2 : 293T cell lysates treated with Okadaic Acid + Calyculin A

Lysates/proteins at 10 µg per lane.

Predicted band size: 73 kDa

Anti-Raf1 (phospho S259) antibody [EPR3433(2)] (ab173539)

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