Product datasheet

Anti-Raf1 antibody ab137435

Overview

<table>
<thead>
<tr>
<th>Product name</th>
<th>Anti-Raf1 antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>Rabbit polyclonal to Raf1</td>
</tr>
<tr>
<td>Host species</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Tested applications</td>
<td>Suitable for: WB, IHC-P, ICC/IF</td>
</tr>
<tr>
<td>Species reactivity</td>
<td>Reacts with: Mouse, Human</td>
</tr>
<tr>
<td></td>
<td>Predicted to work with: Rat, Chicken, Cow, Xenopus tropicalis</td>
</tr>
<tr>
<td>Immunogen</td>
<td>Recombinant fragment corresponding to Human Raf1 (internal sequence). Database link: P04049</td>
</tr>
<tr>
<td>Positive control</td>
<td>WB: 200nM PMA treated (+) HeLa whole cell extracts starved for 24 hours. IHC-P: mouse muscle tissue, human endometrial carcinoma tissue, A549 xenograft. ICC/IF: A549 cells, A431 cells.</td>
</tr>
</tbody>
</table>

Properties

<table>
<thead>
<tr>
<th>Form</th>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage instructions</td>
<td>Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid repeated freeze / thaw cycles.</td>
</tr>
<tr>
<td>Storage buffer</td>
<td>pH: 7.00</td>
</tr>
<tr>
<td></td>
<td>Preservative: 0.025% Proclin 300</td>
</tr>
<tr>
<td></td>
<td>Constituents: PBS, 20% Glycerol</td>
</tr>
<tr>
<td>Purity</td>
<td>Immunogen affinity purified</td>
</tr>
<tr>
<td>Clonality</td>
<td>Polyclonal</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgG</td>
</tr>
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</table>

Applications

Our Abpromise guarantee covers the use of ab137435 in the following tested applications.

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

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tr>
<td>-------------</td>
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<tr>
<td>IHC-P</td>
<td></td>
<td>1/100 - 1/1000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>★★★★★</td>
<td>1/100 - 1/1000.</td>
</tr>
</tbody>
</table>

**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**
All lanes: Anti-Raf1 antibody (ab137435) at 1/1000 dilution

Lane 1: Untreated (−) HeLa whole cell extracts starved for 24 hours
Lane 2: 200nM PMA treated (+) HeLa whole cell extracts starved for 24 hours

Lysates/proteins at 30 µg per lane.

Secondary
All lanes: HRP-conjugated anti-rabbit IgG antibody

Predicted band size: 73 kDa
Additional bands at: 150 kDa (possible dimer)

Raf1 protein may migrate to higher size when phosphorylated and appears as doublet due to different phosphorylation status.

7.5% SDS gel.
Running condition: 80V, 15min; 140V, 40 min.
Transfer condition: Semi-dry, 18 V, 60 min (NC membrane).
Blocking condition: 5% non-fat milk in TBST, RT, 60 min.
Primary antibody incubation: 4°C overnight.
Washing condition: 5 ml TBST, 4 x 5 min.
Exposure: Trident plus Western HRP Substrate.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Raf1 antibody (ab137435)

Immunohistochemical analysis of paraffin-embedded mouse muscle tissue labeling Raf1 with ab137435 at 1/500 dilution. Antigen Retrieval: EDTA based buffer, pH 8.0, 15min. Nuclear staining is observed.

Immunocytochemistry/Immunofluorescence - Anti-Raf1 antibody (ab137435)

Immunocytochemistry/Immunofluorescence analysis A549 cells labeling Raf1 with ab137435 at 1/200 dilution. The cells were fixed with Paraformaldehyde and permeabilized with 0.1% v/v Triton X-100 pH 7.4. CF568 Donkey anti-rabbit IgG(H+L) polyclonal was used as the secondary antibody.

This image is courtesy of an AbReview submitted by Dimitra Kalamida.
Immunohistochemical analysis of formalin-fixed paraffin-embedded human endometrial carcinoma tissue section labeling Raf1 with ab137435 at 1/500.

Immunohistochemical analysis of paraffin-embedded A549 xenograft labelling Raf1 with ab137435 at 1/500 dilution.

Immunofluorescence analysis of paraformaldehyde-fixed A431 cells labelling Raf1 with ab137435 at 1/200 dilution. The image in the lower panel is merged with a DNA probe.

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