abcam

Product datasheet

Anti-BRAF (mutated V600E) antibody [VE1] ab228461

Recombinant

★★★★★ 6 Abreviews 110 References 5 Images

Overview

Product name Anti-BRAF (mutated V600E) antibody [VE1]

Description Mouse monoclonal [VE1] to BRAF (mutated V600E)

Host species Mouse

Specificity The VE1 monoclonal is a sensitive antibody that detects mutated, constitutively active BRAF

protein where glutamic acid is present at codon 600 instead of valine (V600E) (PubMed IDs:

21638088, 23657789).

Please be aware that non-specific nuclear staining has been reported with this antibody (PubMed

IDs: 23763264, 23589031, 24838325).

Tested applications Suitable for: IHC-P, WB

Species reactivity Reacts with: Human

Predicted to work with: Mouse, Chicken

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: A375 cell lysate. IHC-P: A375, Caco-2 and Human melanoma tissue with B-RAF V600E

mutation.

General notes The V600E activating mutation in BRAF is found in several cancer types including: ~65%

of pleomorphic xanthoastrocytomas, ~52% of microsatellite-unstable colon cancer tumors, ~50% of melanomas, ~35% of papillary thyroid carcinomas and ~5% of microsatellite-stable colon

cancers (PubMed IDs: 21274720, 24508103, 18682506, 16024606).

The majority (>90%) of BRAF mutant cancers harbor the V600E mutation. The mutation leads to activation of the MAPK signaling pathway that increases cell invasion and reduces apoptosis. It also leads to reduced expression of melanocyte differentiation antigens and subsequent immune evasion (PubMed IDs: **21638088**, **20551059**).

The VE1 monoclonal antibody is manufactured by Abcam. If you require a particular buffer formulation or a particular conjugate for your experiments, please see **Custom formulation and conjugation services**

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.

1

Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**[®] **patents**.

This product is FOR RESEARCH USE ONLY. For commercial use, please contact partnerships@abcam.com.

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 pH: 7.50

Preservative: 0.1% Sodium azide
Constituents: Tris, 0.3% Carrier protein

Purity Protein A purified

Clonality Monoclonal

Clone numberVE1IsotypeIgG2bLight chain typekappa

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab228461 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
IHC-P	★★★★★ (1)	1/100. Perform heat mediated epitope retrieval with citrate buffer pH6 before commencing with IHC staining protocol
WB	★★★★☆ (2)	1/1000. Predicted molecular weight: 84 kDa.

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 diseaseNote=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.

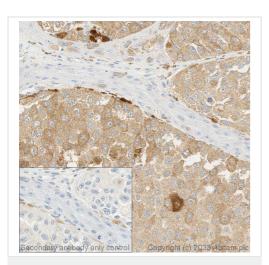
Contains 1 protein kinase domain.

Contains 1 RBD (Ras-binding) domain.

Cellular localization

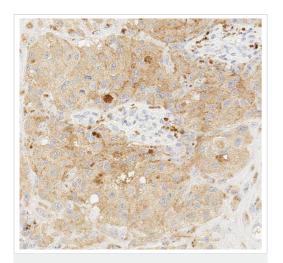
Nucleus. Cytoplasm. Cell membrane. Colocalizes with RGS14 and RAF1 in both the cytoplasm and membranes.

Images



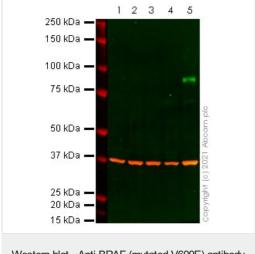
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BRAF (mutated V600E) antibody [VE1] (ab228461)

Immunohistochemical analysis of formalin fixed paraffin embedded human melanoma labelling BRAF (mutated V600E) with ab228461 at 1/600 dilution. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was conducted for 32 mins at 100°C with ULTRA cell conditioning solution (CC1, pH 8.5). ab228461 anti-BRAF (mutated V600E) antibody [VE1] was incubated at 37°C for 16 mins. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BRAF (mutated V600E) antibody [VE1] (ab228461)

Immunohistochemical analysis of formalin-fixed paraffin-embedded human melanoma tissue (carrying mutant BRAF V600E) labelling BRAF (mutated V600E) with ab228461 at 1/100 dilution. The section was pre-treated using heat-mediated antigen retrieval method with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab228461, 1/100 dilution, at room temperature and detected using a Leica BOND™ Polymer refine kit.



Western blot - Anti-BRAF (mutated V600E) antibody [VE1] (ab228461)

All lanes : Anti-BRAF (mutated V600E) antibody [VE1] (ab228461) at 1/1000 dilution

Lane 1 : HCT 116 cell lysate (wildtype BRAF)

Lane 2: SW480 cell lysate (wildtype BRAF)

Lane 3: Caco-2 cell lysate (wildtype BRAF)

Lane 4: HT-29 cell lysate (mutant BRAF V600E)

Lane 5: A375 cell lysate (mutant BRAF V600E)

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 84 kDa **Observed band size:** 85 kDa

False colour image of Western blot: Anti-BRAF (mutated V600E) antibody [VE1] staining at 1/1000 dilution, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] (ab181602) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab228461 was shown to bind specifically to mutant BRAF V600E. 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-Mouse IgG H&L (IRDye® 800CW) preabsorbed (ab216772) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (ab216777) at 1/20000 dilution.

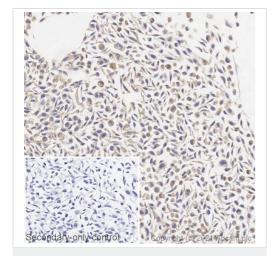
This image was generated in-house using a previous batch, manufactured using hybridoma production method.

IHC image of BRAF (mutated V600E) staining in a section of formalin-fixed paraffin-embedded A375 cell line (carrying mutant BRAF V600E) performed on a Leica BONDTM system using the standard protocol. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab228461, 5ug/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

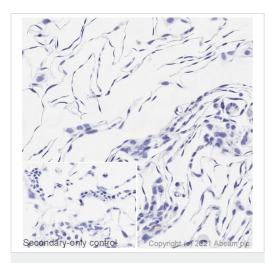
This image was generated in-house using a previous batch, manufactured using hybridoma production method.

Negative control cell line image: IHC image of BRAF (mutated V600E) staining in a section of formalin-fixed paraffin-embedded Caco-2 cell line performed on a Leica BONDTM system using the standard protocol. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab228461, 5ug/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

This image was generated in-house using a previous batch, manufactured using hybridoma production method.



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- We investigate all quality concerns to ensure our products perform to the highest standards

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