abcam

Product datasheet

Anti-Ras (mutated Q61R) antibody [SP174] ab227658

Recombinant RabMAb

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Overview

Product name	Anti-Ras (mutated Q61R) antibody [SP174]
Description	Rabbit monoclonal [SP174] to Ras (mutated Q61R)
Host species	Rabbit
Specificity	This antibody has been found to react with different mutations we tested. We have not tested all possible mutations at Q61.
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: SK-MEL-2 cell lysate, HEK-293T transfected with His-tagged NRAS (mutated Q61R), His-tagged NRAS (mutated Q61K), His-tagged HRAS (mutated Q61R) and His-tagged KRAS (mutated Q61R) whole cell lysates. IHC-P: Human skin melanoma tissue, HEK-293T transfected with NRAS (Q61R) expression vector, HEK-293T transfected with NRAS (Q61K) expression vector, HEK-293T transfected with HRAS (Q61R) expression vector and HEK-293T transfected with KRAS (Q61R) expression vector. Flow Cyt (Intra): SK-MEL-2
General notes	**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: 7.60 Preservative: 0.1% Sodium azide Constituents: PBS, 1% BSA
Purity	Protein A/G purified
Purification notes	Purified from TCS by protein A/G.
Clonality	Monoclonal
Clone number	SP174

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab227658 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)		1/2000.
WB	★★★★★ (1)	1/400 - 1/1000. Predicted molecular weight: 21 kDa.
IHC-P	★★★★★ (1)	1/100. Perform heat mediated antigen retrieval with EDTA buffer pH 8.0 before commencing with IHC staining protocol. This antibody may not be able to detect endogenous NRAS Q61K in patient tissue samples by IHC-P based on a collaborator's data.

Target

Function Ras proteins bind GDP/GTP and possess intrinsic GTPase activity.

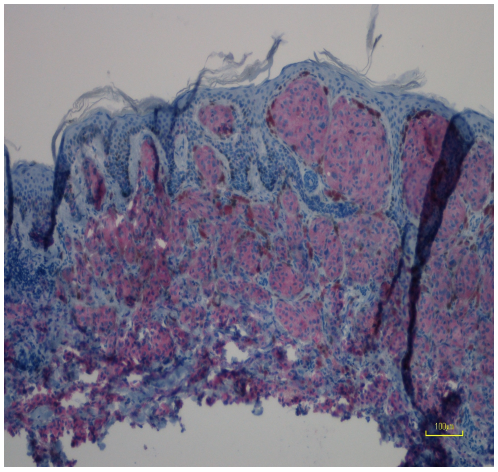
Involvement in disease Defects in HRAS are the cause of faciocutaneoskeletal syndrome (FCSS) [MIM:218040]. A rare condition characterized by prenatally increased growth, postnatal growth deficiency, mental retardation, distinctive facial appearance, cardiovascular abnormalities (typically pulmonic stenosis, hypertrophic cardiomyopathy and/or atrial tachycardia), tumor predisposition, skin and musculoskeletal abnormalities.
Defects in HRAS are the cause of congenital myopathy with excess of muscle spindles (CMEMS) [MIM:218040]. CMEMS is a variant of Costello syndrome.
Defects in HRAS may be a cause of susceptibility to Hurthle cell thyroid carcinoma (HCTC) [MIM:607464]. Hurthle cell thyroid carcinoma accounts for approximately 3% of all thyroid cancers. Although they are classified as variants of follicular neoplasms, they are more often multifocal and somewhat more aggressive and are less likely to take up iodine than are other follicular neoplasms.
Note=Mutations which change positions 12, 13 or 61 activate the potential of HRAS to transform cultured cells and are implicated in a variety of human tumors.
Defects in HRAS are a cause of susceptibility to bladder cancer (BLC) [MIM:109800]. A malignancy originating in tissues of the urinary bladder. It often presents with multiple tumors appearing at different times and at different sites in the bladder. Most bladder cancers are transitional cell carcinomas. They begin in cells that normally make up the inner lining of the bladder. Other types of bladder cancer include squamous cell carcinoma (cancer that begins in thin, flat cells) and adenocarcinoma (cancer that begins in cells that make and release mucus and other fluids). Bladder cancer is a complex disorder with both genetic and environmental influences.
Note=Defects in HRAS are the cause of oral squamous cell carcinoma (OSCC).

Sequence similarities Belongs to the small GTPase superfamily. Ras family.

Post-translational Palmitoylated by the ZDHHC9-GOLGA7 complex. A continuous cycle of de- and re-palmitoylation

modifications	regulates rapid exchange between plasma membrane and Golgi. S-nitrosylated; critical for redox regulation. Important for stimulating guanine nucleotide exchange. No structural perturbation on nitrosylation.
Cellular localization	Cell membrane. Golgi apparatus membrane. The active GTP-bound form is localized most strongly to membranes than the inactive GDP-bound form (By similarity). Shuttles between the plasma membrane and the Golgi apparatus.

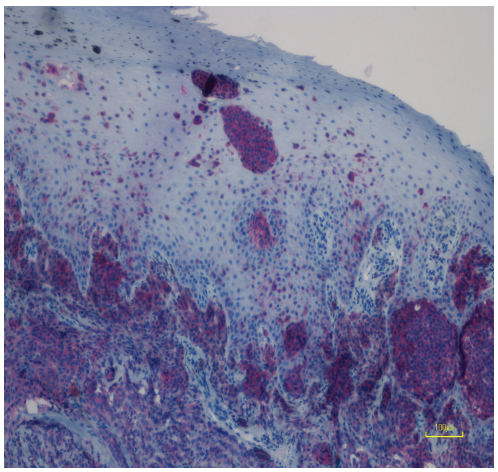
Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ras (mutated Q61R) antibody [SP174] (ab227658)

This image is courtesy of an anonymous collaborator.

Immunohistochemical analysis of paraffin-embedded human primary malignant melanoma tissue (mutation status: NRAS Q61R) labeling NRAS with ab227658 at 1/100 dilution for 30 min at RT. The tissue was counterstained with Hematoxylin. Heat mediated antigen retrieval was achieved by using EDTA buffer pH 9.0 before IHC staining.



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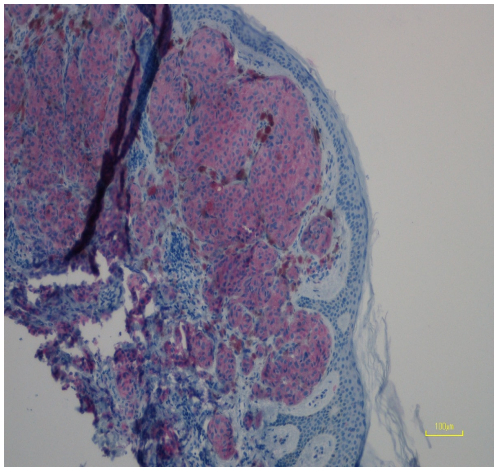

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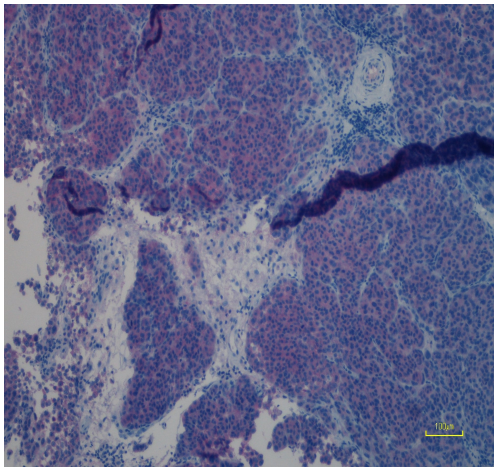
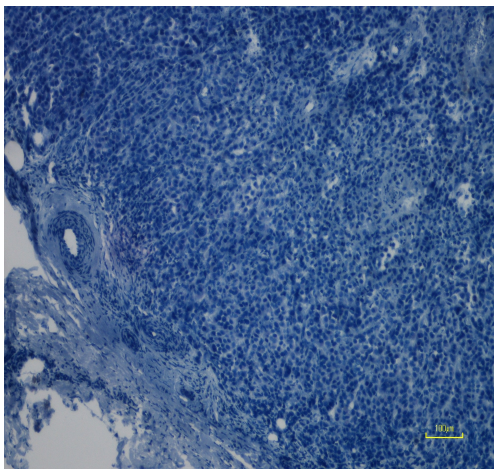


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ras (mutated Q61R) antibody [SP174] (ab227658)

This image is courtesy of an anonymous collaborator.

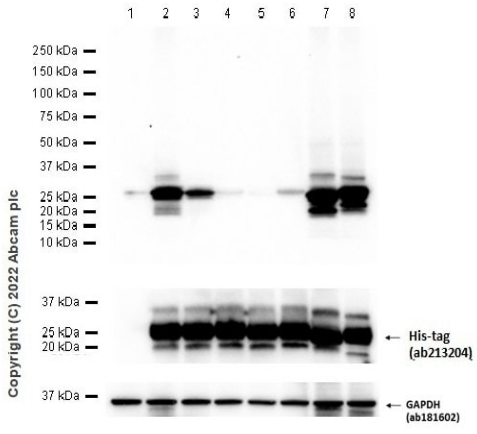
Immunohistochemical analysis of paraffin-embedded human metastatic malignant melanoma tissue (mutation status: NRAS Q61R) labeling NRAS with ab227658 at 1/100 dilution for 30 min at RT. The tissue was counterstained with Hematoxylin. Heat mediated antigen retrieval was achieved by using EDTA buffer pH 9.0 before IHC staining.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ras (mutated Q61R) antibody [SP174] (ab227658)

This image is courtesy of an anonymous collaborator.

Immunohistochemical analysis of paraffin-embedded human metastatic malignant melanoma tissue (mutation status: NRAS Q61K) labeling NRAS with ab227658 at 1/100 dilution for 30 min at RT. The tissue was counterstained with Hematoxylin. Heat mediated antigen retrieval was achieved by using EDTA buffer pH 9.0 before IHC staining.



Western blot - Anti-Ras (mutated Q61R) antibody [SP174] (ab227658)

All lanes : Anti-Ras (mutated Q61R) antibody [SP174] (ab227658) at 1/1000 dilution

Lane 1 : HEK-293T (Human embryonic kidney epithelial cell) transfected with an empty vector (vector control), containing a His-tag, whole cell lysate

Lane 2 : HEK-293T (Human embryonic kidney epithelial cell) transfected with His-tagged NRAS (mutated Q61R) whole cell lysate

Lane 3 : HEK-293T (Human embryonic kidney epithelial cell) transfected with His-tagged NRAS (mutated Q61K) whole cell lysate

Lane 4 : HEK-293T (Human embryonic kidney epithelial cell) transfected with His-tagged NRAS (mutated Q61H) whole cell lysate

Lane 5 : HEK-293T (Human embryonic kidney epithelial cell) transfected with His-tagged NRAS (mutated Q61L) whole cell lysate

Lane 6 : HEK-293T (Human embryonic kidney epithelial cell) transfected with His-tagged NRAS whole cell lysate

Lane 7 : HEK-293T (Human embryonic kidney epithelial cell) transfected with His-tagged HRAS (mutated Q61R) whole cell lysate
Lane 8 : HEK-293T (Human embryonic kidney epithelial cell) transfected with His-tagged KRAS (mutated Q61R) whole cell lysate

Lysates/proteins at 20 µg per lane.

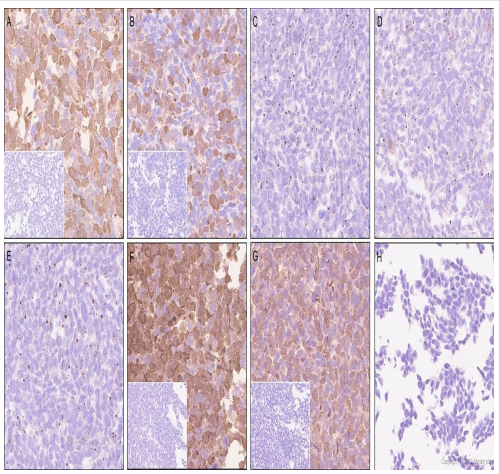
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 21 kDa
Observed band size: 25 kDa

Exposure time: 5 seconds

Blocking buffer and concentration: 5% NFDM/TBST
Diluting buffer and concentration: 5% NFDM /TBST

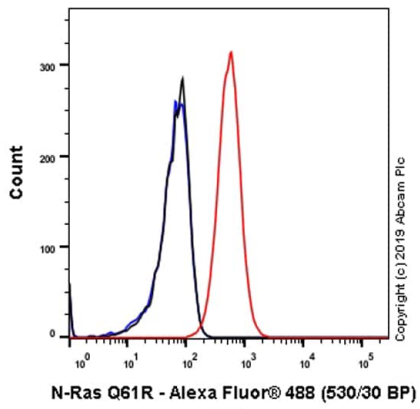
This antibody has been found to react with different mutations we tested. We have not tested all possible mutations at Q61.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ras (mutated Q61R) antibody [SP174] (ab227658)

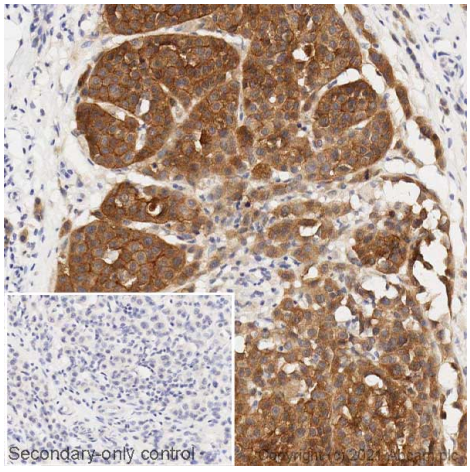
Immunohistochemistry analysis of paraffin-embedded (A) HEK-293T transfected with NRAS (Q61R) expression vector, (B) HEK-293T transfected with NRAS (Q61K) expression vector, (C) HEK-293T transfected with NRAS (Q61H) expression vector, (D) HEK-293T transfected with NRAS (Q61L) expression vector, (E) HEK-293T transfected with NRAS expression vector, (F) HEK-293T transfected with HRAS (Q61R) expression vector, (G) HEK-293T transfected with KRAS(Q61R) and (H) HEK-293T transfected empty vector, sections labelling NRAS (mutated Q61R) with ab227658 at 1/100 dilution. The section was incubated with ab227658 for 10 mins at room temperature. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Sections were counterstained with Hematoxylin. Antigen retrieval was heat mediated using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes.
Positive staining on HEK-293T transfected with NRAS (Q61R) expression vector (Image A), HEK-293T transfected with NRAS (Q61K) expression vector (Image B), HEK-293T transfected with HRAS (Q61R) expression vector (Image F) and HEK-293T

transfected with KRAS (Q61R) expression vector (Image G); Nearly no staining on HEK-293T transfected with NRAS (Q61H) expression vector (Image C), HEK-293T transfected with NRAS (Q61L) expression vector (Image D), HEK-293T transfected with NRAS expression vector (Image E) and HEK-293T transfected empty vector (Image H). The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Flow Cytometry (Intracellular) - Anti-Ras (mutated Q61R) antibody [SP174] (ab227658)

Flow Cytometry analysis of SK-MEL-2(Human skin malignant melanoma) cells labeling NRAS with purifiedab227658 at 1:200 dilution (0.8 µg/ml) (Red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor© 488, **ab150077**) secondary antibody was used at 1:2000 dilution. Isotype control - Rabbit monoclonal IgG (**ab172730**) / Black. Unlabeled control - Unlabelled cells / blue.

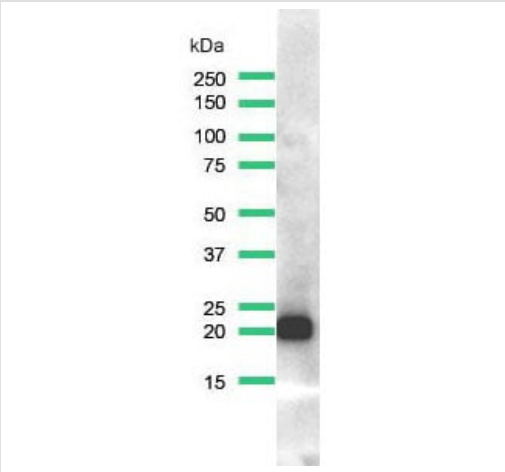


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ras (mutated Q61R) antibody [SP174] (ab227658)

IHC image of NRAS (mutated Q61R) staining in a section of formalin-fixed paraffin-embedded human skin melanoma* performed on a Leica BONDTM system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with EDTA buffer (pH9, epitope retrieval solution 2) for 20mins. The section was then incubated with ab227658, 1/100 dilution, 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.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



Western blot - Anti-Ras (mutated Q61R) antibody [SP174] (ab227658)

Anti-Ras (mutated Q61R) antibody [SP174] (ab227658) at 1/400 dilution + SK-MEL-2 cell lysate

Predicted band size: 21 kDa

Anti-Ras (mutated Q61R) antibody [SP174] (ab227658)

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