abcam

Product datasheet

Anti-Ras (mutated Q61R) antibody [SP174] - BSA and Azide free ab243933

Recombinant RabMAb

10 Images

Overview

Product name	Anti-Ras (mutated Q61R) antibody [SP174] - BSA and Azide free
Description	Rabbit monoclonal [SP174] to Ras (mutated Q61R) - BSA and Azide free
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:** WB, IHC-P, Flow Cyt (Intra)
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	ab243933 is the carrier-free version of **ab227658**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. 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. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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**.

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. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
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 ab243933 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		Use at an assay dependent concentration. Predicted molecular weight: 21 kDa.
IHC-P		Use at an assay dependent concentration. 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.
Flow Cyt (Intra)		Use at an assay dependent concentration.

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.

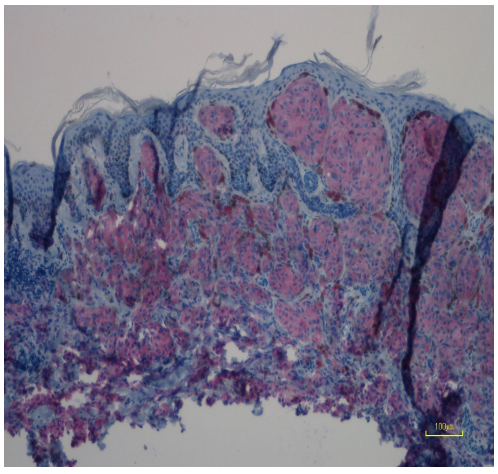
Post-translational modifications

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

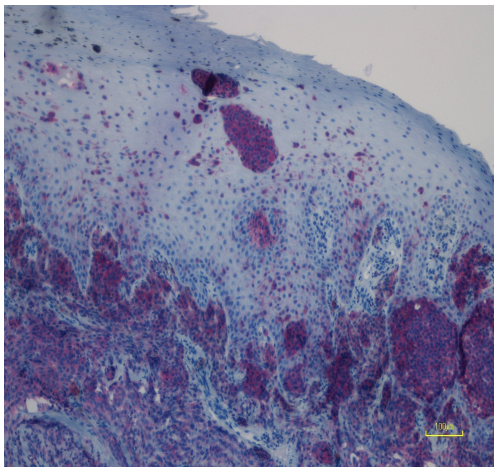
Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ras (mutated Q61R) antibody [SP174] - BSA and Azide free (ab243933)
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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA and sodium azide (**ab227658**).



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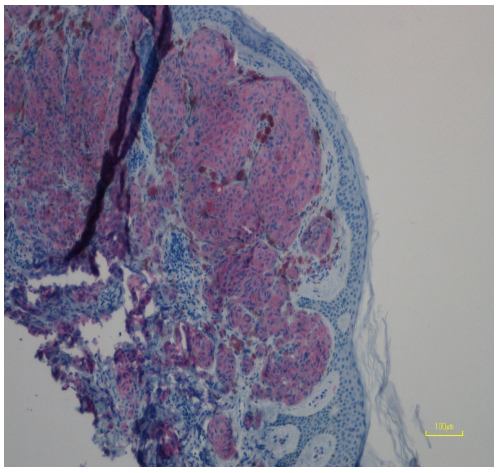

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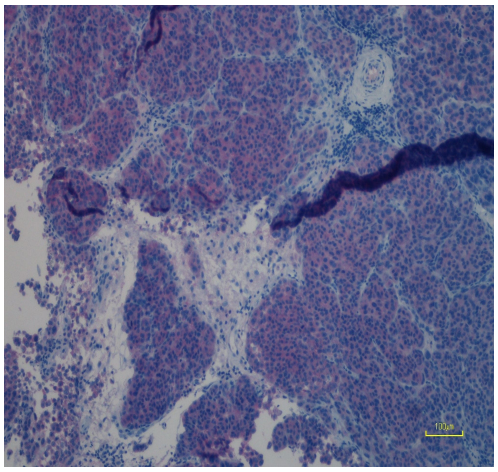
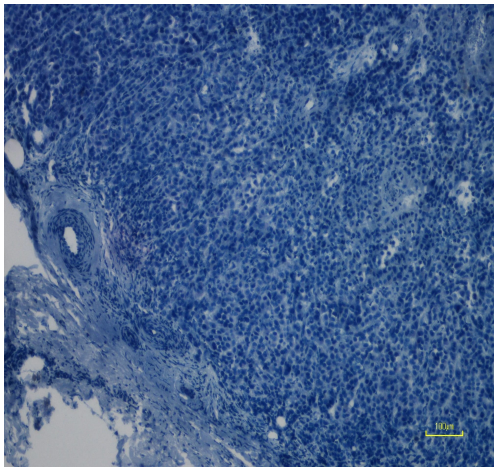


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ras (mutated Q61R) antibody [SP174] - BSA and Azide free (ab243933)
This image is courtesy of an anonymous collaborator.

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.

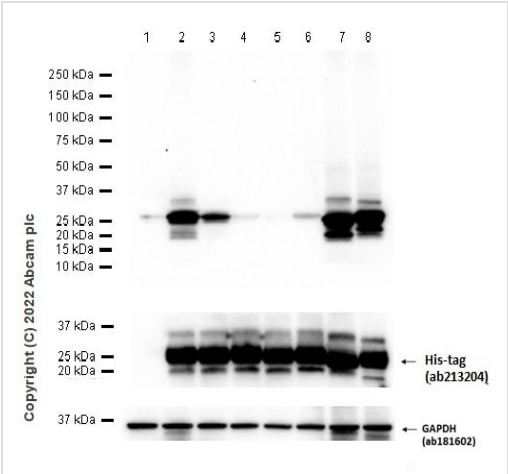
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA and sodium azide (**ab227658**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ras (mutated Q61R) antibody [SP174] - BSA and Azide free (ab243933)
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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA and sodium azide (**ab227658**).



Western blot - Anti-NRAS (mutated Q61R) antibody [SP174] - BSA and Azide free (ab243933)

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

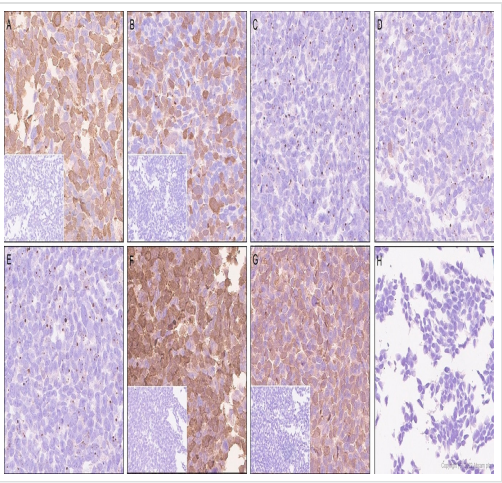
Exposure time: 5 seconds

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.

This data was developed using the same antibody clone in a

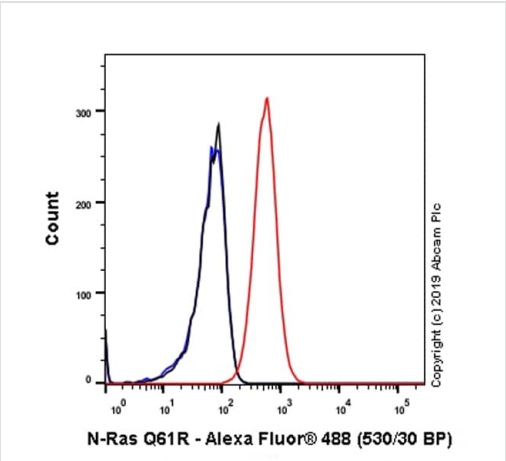
different buffer formulation containing PBS, BSA and sodium azide (**ab227658**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NRAS (mutated Q61R) antibody [SP174] - BSA and Azide free (ab243933)

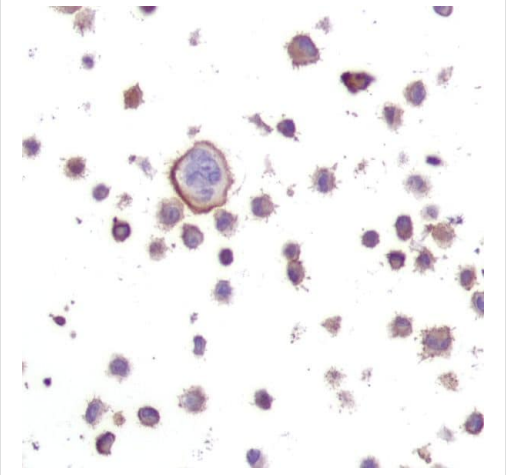
Immunohistochemistry analysis of paraffin-embedded (A) HEK-293T transfected with NRAS (Q61R) expression vector, (B) HEK-293T transfected with NRAS (Q61K) expression vector, (C) HEK-293T transfected with NRAS (Q61H) expression vector, (D) HEK-293T transfected with NRAS (Q61L) expression vector, (E) HEK-293T 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227658**).



Flow Cytometry (Intracellular) - Anti-NRAS (mutated Q61R) antibody [SP174] - BSA and Azide free (ab243933)

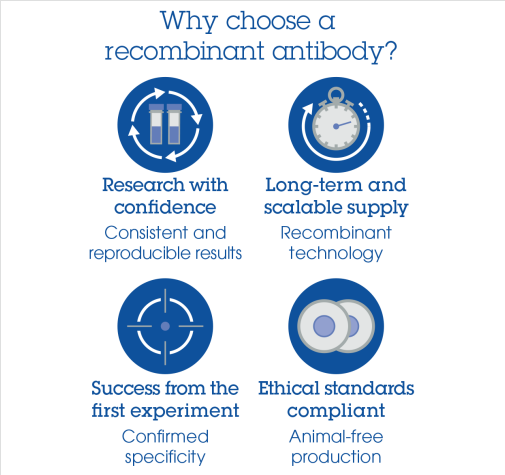
Intracellular Flow Cytometry analysis of SK-MEL-2 (Human skin malignant melanoma) cells labeling NRAS with purified**ab227658** at 1/200 dilution (0.8µg/ml) (Red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor© 488, **ab150077**) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (**ab172730**) / Black. Unlabeled control - Unlabelled cells / blue. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab227658**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NRAS (mutated Q61R) antibody [SP174] - BSA and Azide free (ab243933)

Formalin-fixed, paraffin-embedded human SK-MEL-2 cells stained for NRAS (mutated Q61R) using **ab227658** at 1/100 dilution in immunohistochemical analysis.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA and sodium azide (**ab227658**).



Anti-NRAS (mutated Q61R) antibody [SP174] - BSA and Azide free (ab243933)

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