abcam

Product datasheet

Anti-HRAS + KRAS antibody [EPR18713] ab191595

Recombinant RabMAb

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Overview

Product name Anti-HRAS + KRAS antibody [EPR18713]

Description Rabbit monoclonal [EPR18713] to HRAS + KRAS

Host species Rabbit

Specificity Immunoprecipitation requires a mild lysis buffer to achieve an acceptable enrichment.

Tested applications Suitable for: ICC/IF, WB, IP

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Human KRAS and HRAS full length proteins; Human fetal kidney and fetal brain lysates;

Mouse and Rat brain lysates; A549, C6, A431 and RAW 264.7 whole cell lysates. ICC/iF: HeLa

and NIH/3T3 cells. IP: A549 whole cell lysate.

General notesThis 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 pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

1

Clone number EPR18713

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab191595 in the following tested applications.

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

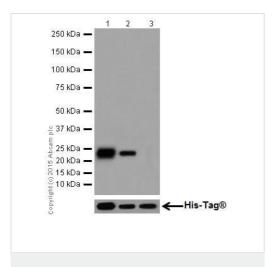
Application	Abreviews	Notes
ICC/IF		1/500.
WB	★★★★★ (5)	1/2000. Detects a band of approximately 21 kDa (predicted molecular weight: 22, 21 kDa).
IP	★ ★ ★ ★ ★ (2)	1/70. Immunoprecipitation requires a mild lysis buffer to achieve an acceptable enrichment.

Target

Relevance

KRAS: Ras proteins bind GDP/GTP and possess intrinsic GTPase activity. Plays an important role in the regulation of cell proliferation (PubMed:23698361, PubMed:22711838). Enzyme regulation: Alternates between an inactive form bound to GDP and an active form bound to GTP. Activated by a guanine nucleotide-exchange factor (GEF) and inactivated by a GTPase-activating protein (GAP). Interaction with SOS1 promotes exchange of bound GDP by GTP. HRAS: Ras proteins bind GDP/GTP and possess intrinsic GTPase activity. Enzyme regulation: Alternates between an inactive form bound to GDP and an active form bound to GTP. Activated by a guanine nucleotide-exchange factor (GEF) and inactivated by a GTPase-activating protein (GAP).

Images



Western blot - Anti-HRAS + KRAS antibody [EPR18713] (ab191595) **All lanes :** Anti-HRAS + KRAS antibody [EPR18713] (ab191595) at 1/10000 dilution

Lane 1 : Human KRAS full length protein
Lane 2 : Human HRAS full length protein
Lane 3 : Human NRAS full length protein

Lysates/proteins at 0.01 µg per lane.

Secondary

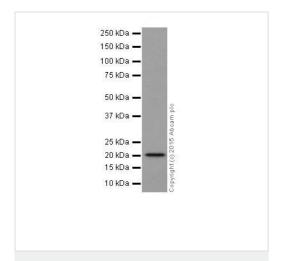
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/50000 dilution

Predicted band size: 22, 21 kDa Observed band size: 21 kDa

Exposure time: 1 second

Blocking/Dilution buffer: 5% NFDM/TBST.

Human KRAS full length protein contains aa1-189 with His-Tag® at N-Terminus; Human HRAS full length protein contains aa1-189 with His-Tag® at N-Terminus; Human NRAS full length protein contains aa1-189 with His-Tag® at N-Terminus. These are all made inhouse.



Western blot - Anti-HRAS + KRAS antibody [EPR18713] (ab191595) Anti-HRAS + KRAS antibody [EPR18713] (ab191595) at 1/2000 dilution + Human fetal kidney lysate at 10 µg

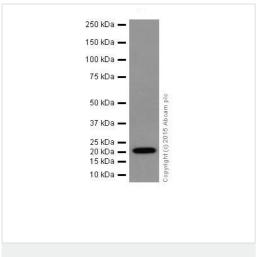
Secondary

Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG at 1/50000 dilution

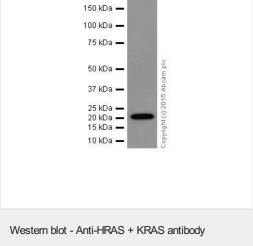
Predicted band size: 22, 21 kDa **Observed band size:** 21 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



[EPR18713] (ab191595)



250 kDa • 150 kDa -100 kDa 🗕 75 kDa 🕳 50 kDa -37 kDa -25 kDa 🕳 20 kDa -15 kDa -10 kDa -

Western blot - Anti-HRAS + KRAS antibody [EPR18713] (ab191595)

Anti-HRAS + KRAS antibody [EPR18713] (ab191595) at 1/5000 dilution + Human fetal brain lysate at 10 µg

Secondary

Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG at 1/50000 dilution

Predicted band size: 22, 21 kDa Observed band size: 21 kDa

Exposure time: 5 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

All lanes: Anti-HRAS + KRAS antibody [EPR18713] (ab191595) at 1/2000 dilution

Lane 1: Mouse brain lysate Lane 2: Rat brain lysate

Lysates/proteins at 10 µg per lane.

Secondary

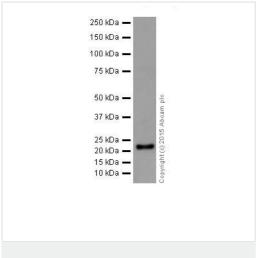
All lanes: Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/50000

dilution

Predicted band size: 22, 21 kDa Observed band size: 21 kDa

Exposure time: 5 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Anti-HRAS + KRAS antibody [EPR18713] (ab191595) at 1/2000 dilution + A549 (Human lung carcinoma) whole cell lysate at 10 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 22, 21 kDa

Observed band size: 21 kDa

Exposure time: 3 minutes

У

Western blot - Anti-HRAS + KRAS antibody [EPR18713] (ab191595)

Blocking/Dilution buffer: 5% NFDM/TBST.

All lanes : Anti-HRAS + KRAS antibody [EPR18713] (ab191595) at 1/5000 dilution

Lane 1: C6 (Rat glial tumor cells) whole cell lysate

Lane 2: A431 (Human epidermoid carcinoma) whole cell lysate

Lane 3: RAW 264.7 (Mouse macrophage cells transformed with

Abelson murine leukemia virus) whole cell lysate

Lysates/proteins at 10 µg per lane.

1 2 3 250 kDa — 150 kDa — 100 kDa — 75 kDa — 37 kDa — 25 kDa — 20 kDa — 15 kDa — 10 kDa — 10 kDa —

Western blot - Anti-HRAS + KRAS antibody [EPR18713] (ab191595)

Secondary

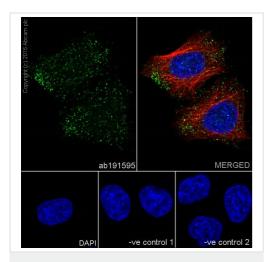
All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000

dilution

Predicted band size: 22, 21 kDa **Observed band size:** 21 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-HRAS + KRAS antibody [EPR18713] (ab191595)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling KRAS + HRAS with ab191595 at 1/500 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

Confocal image showing cytoplasm and nuclear staining on HeLa cell line

The nuclear counterstain is DAPI (blue).

Tubulin is detected with <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution and <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab191595 at 1/500 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution. -ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/1000 dilution.

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DAPI -ve control 1 -ve control 2

Immunocytochemistry/ Immunofluorescence - Anti-HRAS + KRAS antibody [EPR18713] (ab191595)

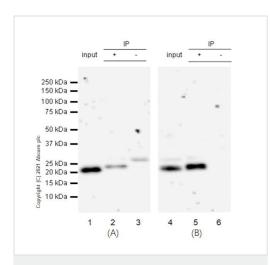
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embyro fibroblast cells) cells labeling KRAS + HRAS with ab191595 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

Confocal image showing cytoplasm and nuclear staining on NIH/3T3 cells line.

The nuclear counterstain is DAPI (blue). Tubulin is detected with <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution and <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab191595 at 1/500 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/1000 dilution. -ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/1000 dilution.



Immunoprecipitation - Anti-HRAS + KRAS antibody [EPR18713] (ab191595)

Immunoprecipitation analysis shows milder lysis buffer is required to get desirable enrichment.

Panel A: RIPA buffer

Panel B: IP lysis buffer (see protocol for details)

HRAS + KRAS was immunoprecipitated from Hepa1-6 (Mouse hepatoma epithelial cell) whole cell lysate 10µg with ab191595 at 1/30 dilution (2ug). Western blot was performed on the immunoprecipitate using ab191595 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/5000 dilution.

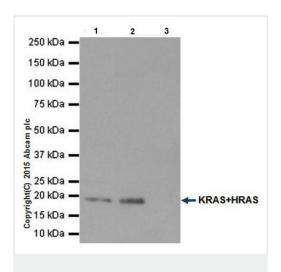
Lane 1 & 4: Hepa1-6 (Mouse hepatoma epithelial cell) whole cell lysate 10µg

Lane 2 & 5: ab191595 IP in Hepa1-6 whole cell lysate

 $\mbox{ {\bf Lane 3\&6:} Rabbit monoclonal lgG} \ (\mbox{{\bf \underline{ab172730}}}) \ \mbox{ instead of ab191595 in Hepa1-6 whole cell} \\ \mbox{lysate}$

Blocking and dilution buffer and concentration: 5% NFDMTBST.

Exposure time: 3 minutes



Immunoprecipitation - Anti-HRAS + KRAS antibody [EPR18713] (ab191595)

KRAS was immunoprecipitated from 1mg of A549 (Human lung carcinoma) whole cell lysate with ab191595 at 1/70 dilution.

Western blot was performed from the immunoprecipitate using ab191595 at 1/1000 dilution.

Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG, was used as secondary antibody at 1/1500 dilution.

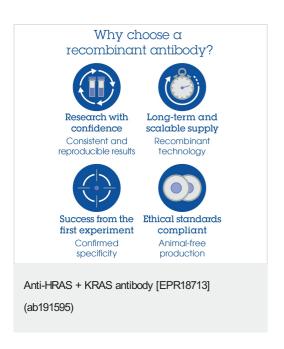
Lane 1: A549 whole cell lysate 10ug (Input).

Lane 2: ab191595 IP in A549 whole cell lysate.

Lane 3: Rabbit monoclonal lgG ($\underline{ab172730}$) instead of ab191595 in A549 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 10 seconds.



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