abcam

Product datasheet

Human NRAS knockout HEK-293T cell line ab266684

4 Images

Overview

Product name	Human NRAS knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 5 bp deletion in exon 2 and 8 bp deletion in exon 2
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	**Recommended control:** Human wild-type HEK293T cell line (**ab255449**). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** DMEM (High Glucose) + 10% FBS **Initial handling guidelines:** Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability. 1. Thaw the vial in 37°C water bath for approximately 1-2 minutes. 2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution. 3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of $2x10^4$ cells/cm^2. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37°C incubator with 5% CO_2. Cultures should be monitored daily. **Subculture guidelines:** All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $2x10^4$ cells/cm^2 is recommended. A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.

Cells should be passaged when they have achieved 80-90% confluence.
This product is subject to limited use licenses from The Broad Institute and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant patents please refer to our **limited use license** and **patent pages**.

We will provide viable cells that proliferate on revival.

Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Kidney
Cell type	epithelial
STR Analysis	Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01: 7, 9.3 TPOX: 11 CSF1PO: 11, 12
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

Target

Function	Ras proteins bind GDP/GTP and possess intrinsic GTPase activity.
Involvement in disease	Defects in NRAS are a cause of juvenile myelomonocytic leukemia (JMML) [MIM:607785]. JMML is a pediatric myelodysplastic syndrome that constitutes approximately 30% of childhood cases of myelodysplastic syndrome (MDS) and 2% of leukemia. Defects in NRAS are the cause of Noonan syndrome type 6 (NS6) [MIM:613224]. A syndrome 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.
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.
Cellular localization	Cell membrane. Golgi apparatus membrane. Shuttles between the plasma membrane and the Golgi apparatus.

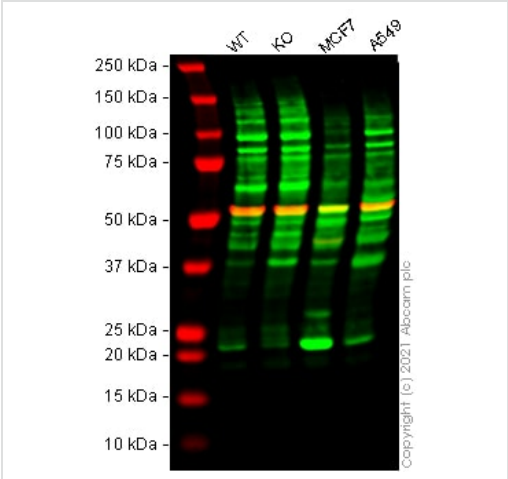
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab266684 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.

Images



Western blot - Human NRAS knockout HEK-293T cell line (ab266684)

All lanes : Anti-NRAS antibody - C-terminal (**ab198820**) at 1/200 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : NRAS knockout HEK-293T cell lysate
Lane 3 : MCF7 cell lysate
Lane 4 : A549 cell lysate

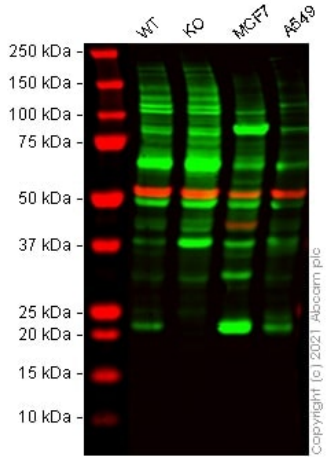
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

Lanes 1 - 4: Merged signal (red and green). Green - **ab198820** observed at 22 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab198820 was shown to react with NRAS in wild-type HEK-293T cells in Western blot with loss of signal observed in NRAS knockout cell line ab266684 (NRAS knockout cell lysate **ab258542**). Wild-type HEK-293T and NRAS knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab198820** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1 in 200 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Human NRAS knockout HEK-293T cell line (ab266684)

All lanes : Anti-NRAS antibody (**ab167136**) at 0.5 µg/ml

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : NRAS knockout HEK-293T cell lysate
Lane 3 : MCF7 cell lysate
Lane 4 : A549 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

Lanes 1 - 4: Merged signal (red and green). Green - **ab167136** observed at 22 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab167136 was shown to react with NRAS in wild-type HEK-293T cells in Western blot with loss of signal observed in NRAS knockout cell line ab266684 (NRAS knockout cell lysate **ab258542**). Wild-type HEK-293T and NRAS knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab167136** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at 0.5 µg/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.

Sanger Sequencing - Human NRAS knockout

HEK293T cell line (ab266684)

Allele-1: 8 bp deletion in exon 2

Sanger Sequencing - Human NRAS knockout

HEK293T cell line (ab266684)

Allele-2: 5 bp deletion in exon 2.

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