Anti-KRAS Antibody [A8E5]

HA601059

Product Type:	Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 22 kDa
Clone number:	A8E5

Description: The K-Ras protein is a GTPase, a class of enzymes which convert the nucleotide guanosine triphosphate (GTP) into guanosine diphosphate (GDP). In this way the K-Ras protein acts like a switch that is turned on and off by the GTP and GDP molecules. To transmit signals, it must be turned on by attaching (binding) to a molecule of GTP. The K-Ras protein is turned off (inactivated) when it converts the GTP to GDP. When the protein is bound to GDP, it does not relay signals to the cell's nucleus. Several germline KRAS mutations have been found to be associated with Noonan syndrome and cardio-facio-cutaneous syndrome. Somatic KRAS mutations are found at high rates in leukemias, colorectal cancer, pancreatic cancer and lung cancer. KRAS mutations are more commonly observed in cecal cancers than colorectal cancers located in any other places from ascending colon to rectum. KRAS gene can also be amplified in colorectal cancer. Tumors or cell lines harboring this genetic lesion are not responsive to EGFR inhibitors. Although KRAS amplification is an infrequent event in colorectal cancer, it might be responsible for precluding response to anti-EGFR treatment in some patients. Amplification of wild-type Kras has also been observed in ovarian, gastric, uterine, and lung cancers. Driver mutations in KRAS underlie the pathogenesis of up to 20% of human cancers. Hence KRAS is an attractive drug target, however lack of obvious binding sites has hindered pharmaceutical development. One potential drug interaction site is where GTP/GDP binds. However, due to the extraordinarily high affinity of GTP/GDP for this site, it is unlikely that drug-like small molecule inhibitors could compete with GTP/GDP binding. Other than where GTP/GDP binds, there are no obvious high affinity binding sites for small molecules.

Immunogen: Recombinant protein within human KRAS aa 2-186.

Positive control: HCT 116 cell lysate, HEK-293 cell lysate, 22RV1 cell lysate, Mouse brain tissue lysate, Mouse kidney tissue lysate, Rat brain tissue lysate, Jurkat, NIH/3T3, human lung carcinoma tissue.

Subcellular location: Cell membrane. Cytoplasm.

Database links: SwissProt: P01116 Human | P32883 Mouse | P08644 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:100
IHC-P	1:1100
FC	1:500-1:1,000

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

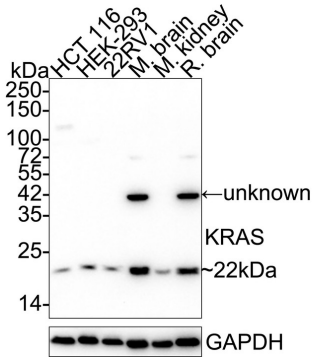

Fig1: Western blot analysis of KRAS on different lysates with Mouse anti-KRAS antibody (HA601059) at 1/2,000 dilution.

Lane 1: HCT 116 cell lysate
Lane 2: HEK-293 cell lysate
Lane 3: 22RV1 cell lysate
Lane 4: Mouse brain tissue lysate
Lane 5: Mouse kidney tissue lysate
Lane 6: Rat brain tissue lysate

Lysates/proteins at 30 µg/Lane.

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

Exposure time: 3 minutes; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA601059) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

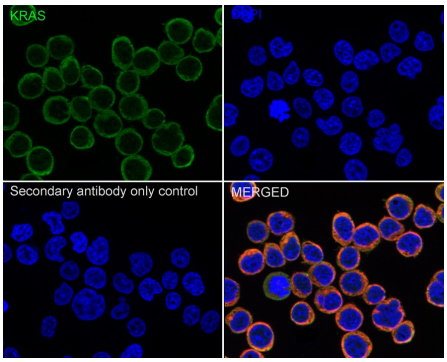

Fig2: Immunocytochemistry analysis of Jurkat cells labeling KRAS with Mouse anti-KRAS antibody (HA601059) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Mouse anti-KRAS antibody (HA601059) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

beta Tubulin (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

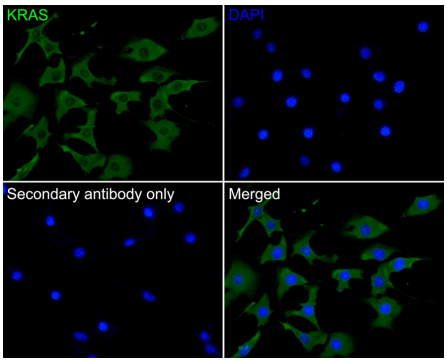

Fig3: Immunocytochemistry analysis of NIH/3T3 cells labeling KRAS with Mouse anti-KRAS antibody (HA601059) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 30 minutes, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes, and then blocked with 2% BSA for 30 minutes at room temperature. Cells were then incubated with Mouse anti-KRAS antibody (HA601059) at 1/100 dilution in 2% BSA overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

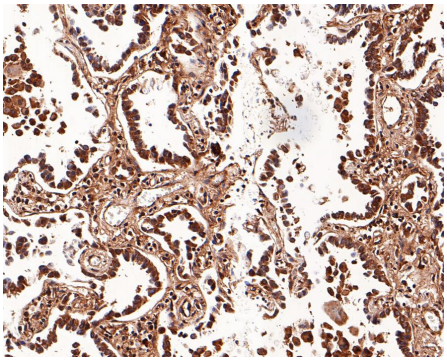

Fig4: Immunohistochemical analysis of paraffin-embedded human lung carcinoma tissue with Mouse anti-KRAS antibody (HA601059) at 1/1,100 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA601059) at 1/1,100 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

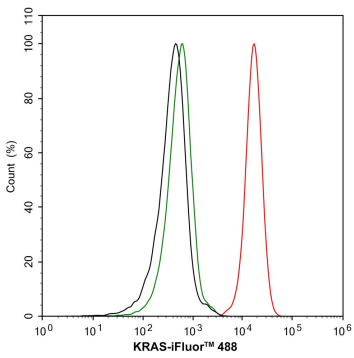

Fig5: Flow cytometric analysis of Jurkat cells labeling KRAS.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA601059, 1/1,000) (red) compared with Mouse IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Mouse IgG Secondary antibody (HA1125) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

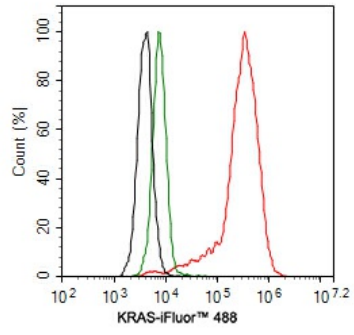

Fig6: Flow cytometric analysis of NIH/3T3 cells labeling KRAS.

Cells were fixed and permeabilized, and then blocked with 2% negative goat serum for 15 minutes at room temperature.Then stained with the primary antibody (HA601059, 1ug/ml) (red) compared with Mouse IgG1 Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Mouse IgG Secondary antibody (HA1125) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Christophe Rosty et al. Colorectal carcinomas with KRAS mutation are associated with distinctive morphological and molecular features. Mod Pathol. 2013 Jun;26(6):825-34. doi: 10.1038/modpathol.2012.240. Epub 2013 Jan 25.
2. N Tsuchida, T Ryder, E Ohtsubo. Nucleotide sequence of the oncogene encoding the p21 transforming protein of Kirsten murine sarcoma virus. Science. 1982 Sep 3;217(4563):937-9. doi: 10.1126/science.6287573.