Anti-Ras Antibody [JF10-11]

ET1702-94

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Zebrafish
Applications:	WB, IF-Cell, IP, FC
Molecular Wt:	Predicted band size: 21 kDa
Clone number:	JF10-11

Description: The mammalian c-H-, c-K- and N-Ras proto-oncogenes encode guanine nucleotide-binding proteins that are ubiquitously expressed in vertebrate cells. c-H- and c-K-Ras are cellular homologs of the v-H and v-K-Ras sequences originally isolated from the Harvey and Kirsten strains of rat sarcoma virus. Ras-encoded proteins bind GDP and GTP with high affinity and possess a low level intrinsic GTPase activity that can be stimulated over 100-fold by interaction with cytosolic GTPase activating protein (GAP), a potential effector for Ras p21 function. Point mutations at amino acids 12, 13, 59 and 61 within domains responsible for GTP binding and hydrolysis activate Ras proteins to their oncogenic form and block the ability of the GTPase activity to be stimulated by GAP. Several additional proteins with GAP activity have been identified and shown to interact with p21 Ras or other members of the Ras gene family.

Immunogen: Synthetic peptide within Human Ras aa 21-64 / 189.

Positive control: MCF7 cell lysate, HeLa cell lysate, NIH/3T3 cell lysate, Mouse brain tissue lysate, Mouse ovary tissue lysate, Rat brain tissue lysate, 293T cell lysate, Zebrafish tissue lysate, HeLa, PC-12.

Subcellular location: Cytoplasm, Cell membrane, Golgi apparatus, Nucleus.

Database links: SwissProt: P01111 Human | P01112 Human | P01116 Human | P08556 Mouse | P32883 Mouse | Q61411 Mouse | P08644 Rat | P20171 Rat | Q04970 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
IF-Cell	1:100-1:500
IP	Use at an assay dependent concentration.
FC	1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% 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

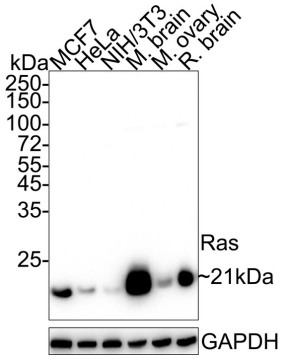

Fig1: Western blot analysis of Ras on different lysates with Rabbit anti-Ras antibody (ET1702-94) at 1/1,000 dilution.

Lane 1: MCF7 cell lysate (15 µg/Lane)
Lane 2: HeLa cell lysate (15 µg/Lane)
Lane 3: NIH/3T3 cell lysate (15 µg/Lane)
Lane 4: Mouse brain tissue lysate (30 µg/Lane)
Lane 5: Mouse ovary tissue lysate (30 µg/Lane)
Lane 6: Rat brain tissue lysate (30 µg/Lane)

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

Exposure time: 25 seconds; 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 (ET1702-94) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

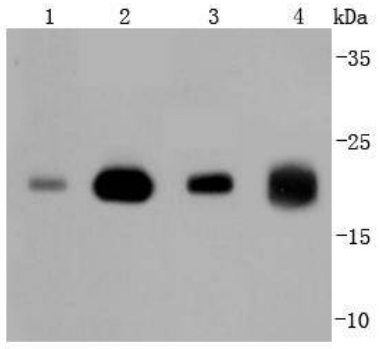

Fig2: Western blot analysis of Ras on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1702-94, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.

Positive control:

Lane 1: 293T cell lysate
Lane 2: MCF7 cell lysate
Lane 3: HeLa cell lysate
Lane 4: Zebrafish tissue lysate

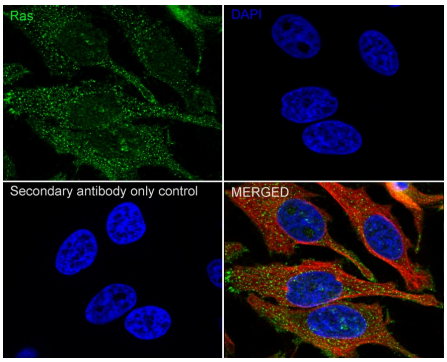

Fig3: Immunocytochemistry analysis of HeLa cells labeling Ras with Rabbit anti-Ras antibody (ET1702-94) at 1/100 dilution.

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 Rabbit anti-Ras antibody (ET1702-94) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) 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 (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

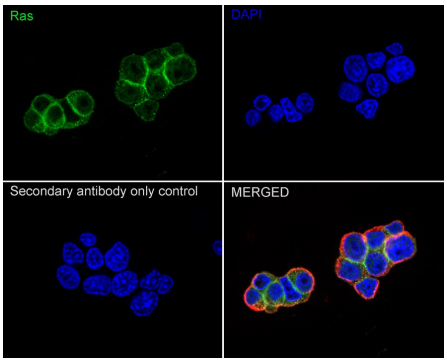

Fig4: Immunocytochemistry analysis of PC-12 cells labeling Ras with Rabbit anti-Ras antibody (ET1702-94) at 1/100 dilution.

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 Rabbit anti-Ras antibody (ET1702-94) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) 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 (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

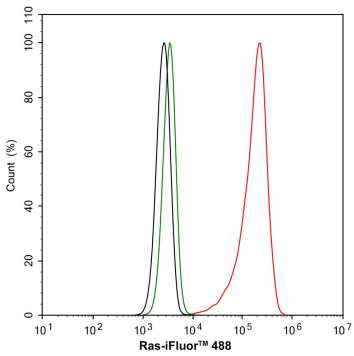

Fig5: Flow cytometric analysis of HeLa cells labeling Ras.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1702-94, 1/1,000) (red) compared with Rabbit 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-Rabbit IgG Secondary antibody (HA1121) 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. Zhou M et al. VPS35 binds farnesylated N-Ras in the cytosol to regulate N-Ras trafficking. J Cell Biol 214:445-58 (2016).
2. Meng X et al. RPL23 Links Oncogenic RAS Signaling to p53-Mediated Tumor Suppression. Cancer Res 76:5030-9 (2016).