Anti-Ras Antibody [SA39-05]

ET1601-16

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Zebrafish
Applications:	WB, IF-Cell, FC, IP
Molecular Wt:	Predicted band size: 21 kDa
Clone number:	SA39-05

Description: Ras, from "Rat sarcoma virus", is a family of related proteins that are expressed in all animal cell lineages and organs. All Ras protein family members belong to a class of protein called small GTPase, and are involved in transmitting signals within cells (cellular signal transduction). Ras is the prototypical member of the Ras superfamily of proteins, which are all related in three-dimensional structure and regulate diverse cell behaviours. When Ras is 'switched on' by incoming signals, it subsequently switches on other proteins, which ultimately turn on genes involved in cell growth, differentiation, and survival. Mutations in Ras genes can lead to the production of permanently activated Ras proteins, which can cause unintended and overactive signaling inside the cell, even in the absence of incoming signals. Because these signals result in cell growth and division, overactive Ras signaling can ultimately lead to cancer. The three Ras genes in humans (HRAS, KRAS, and NRAS) are the most common oncogenes in human cancer.

Immunogen: Synthetic peptide within Human Ras aa 7-56 / 189.

Positive control: 293T cell lysate, MCF7 cell lysate, NIH/3T3 cell lysate, Neuro-2a cell lysate, PC-12 cell lysate, Mouse brain tissue lysate, MCF7, NIH/3T3, PC-12.

Subcellular location: Cytoplasm, Cell membrane, Golgi apparatus.

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
FC	1:1,000
IP	1-2μg/sample

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

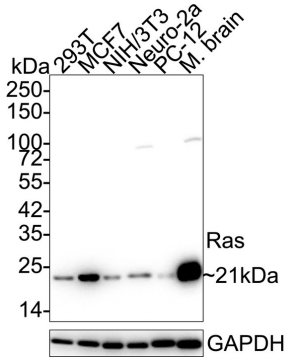

Fig1: Western blot analysis of Ras on different lysates with Rabbit anti-Ras antibody (ET1601-16) at 1/1,000 dilution.

Lane 1: 293T cell lysate (10 µg/Lane)
Lane 2: MCF7 cell lysate (10 µg/Lane)
Lane 3: NIH/3T3 cell lysate (10 µg/Lane)
Lane 4: Neuro-2a cell lysate (10 µg/Lane)
Lane 5: PC-12 cell lysate (10 µg/Lane)
Lane 6: Mouse brain tissue lysate (20 µg/Lane)

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

Exposure time: 30 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 (ET1601-16) 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.

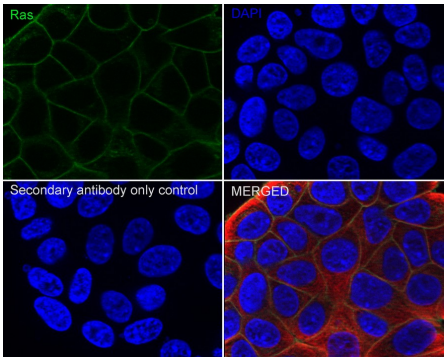

Fig2: Immunocytochemistry analysis of MCF7 cells labeling Ras with Rabbit anti-Ras antibody (ET1601-16) at 1/100 dilution.

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 (ET1601-16) 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.

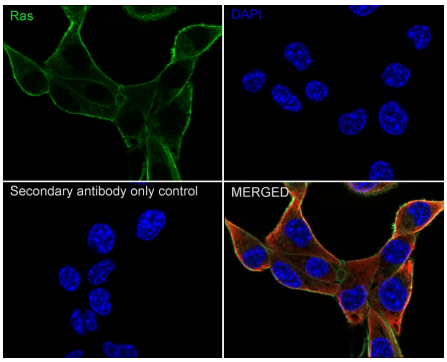

Fig3: Immunocytochemistry analysis of NIH/3T3 cells labeling Ras with Rabbit anti-Ras antibody (ET1601-16) at 1/100 dilution.

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 (ET1601-16) 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.

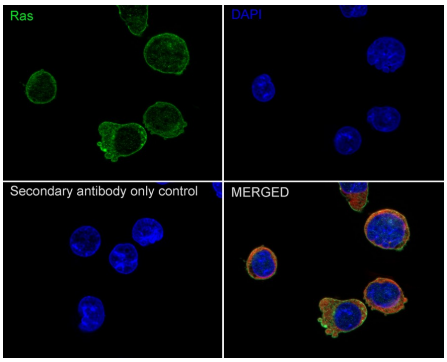

Fig4: Immunocytochemistry analysis of PC-12 cells labeling Ras with Rabbit anti-Ras antibody (ET1601-16) at 1/100 dilution.

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 (ET1601-16) 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.

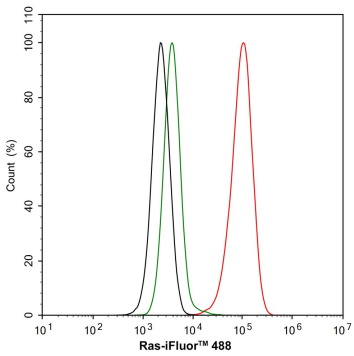

Fig5: Flow cytometric analysis of MCF7 cells labeling Ras.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1601-16, 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).

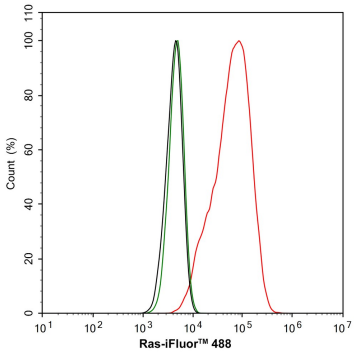

Fig6: Flow cytometric analysis of NIH/3T3 cells labeling Ras.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1601-16, 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).

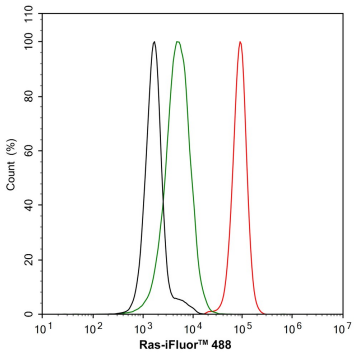

Fig7: Flow cytometric analysis of PC-12 cells labeling Ras.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1601-16, 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).

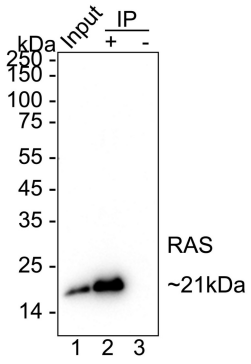

Fig8: Ras was immunoprecipitated from 0.2 mg MCF7 cell lysate with ET1601-16 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using ET1601-16 at 1/2,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: MCF7 cell lysate (input)
Lane 2: ET1601-16 IP in MCF7 cell lysate
Lane 3: Rabbit IgG instead of ET1601-16 in MCF7 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 10 seconds; ECL: K1801

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. "NRAS mutation causes a human autoimmune lymphoproliferative syndrome." Oliveira J.B. Bidere N. Niemela J.E. Zheng L. Sakai K., Nix C.P. Danner R.L. Barb J., Munson P.J. Puck J.M. Dale J. Straus S.E. Fleisher T.A. Lenardo M.J. Proc. Natl. Acad. Sci. U.S.A. 104:8953-8958(2007)