

# Anti-Ras Antibody

## ER40115



<b>Product Type:</b>	Rabbit polyclonal IgG, primary antibodies
<b>Species reactivity:</b>	Human, Mouse, Rat
<b>Applications:</b>	WB, IF-Cell, IHC-P, FC
<b>Molecular Wt:</b>	Predicted band size: 21 kDa

**Description:** Ras superfamily is a protein superfamily of small GTPases, which are all related, to a degree, to the Ras protein subfamily (the key human members of which are KRAS, NRAS, and HRAS). Receptor tyrosine kinases and G protein-coupled receptors activate Ras, which then stimulates the Raf-MEK-MAPK pathway. GTPase-activating proteins (GAP) normally facilitate the inactivation of Ras. However, research studies have shown that in 30% of human tumors, point mutations in Ras prevent the GAP-mediated inhibition of this pathway. The most common oncogenic Ras mutation found in tumors is Gly12 to Asp12 (G12D), which prevents Ras inactivation, possibly by increasing the overall rigidity of the protein. This antibody is predicted to react with H-Ras, N-Ras and K-Ras.

**Immunogen:** Synthetic peptide within Human Ras aa 11-60 / 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, HeLa, PC-12, rat small intestine tissue, human liver cancer tissue, human kidney muscle tissue, mouse kidney tissue, mouse small intestine tissue.

**Subcellular location:** Cytoplasmic side, cell membrane, Membrane.

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

**Recommended Dilutions:**

<b>WB</b>	1:1,000
<b>IF-Cell</b>	1:100
<b>IHC-P</b>	1:200
<b>FC</b>	1:1,000

**Storage Buffer:** 1\*PBS (pH7.4), 0.2% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

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

**Purity:** Immunogen affinity purified.

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Orders:0086-571-88062880

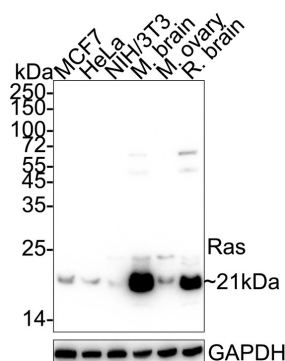
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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

**Fig1:** Western blot analysis of Ras on different lysates with Rabbit anti-Ras antibody (ER40115) 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

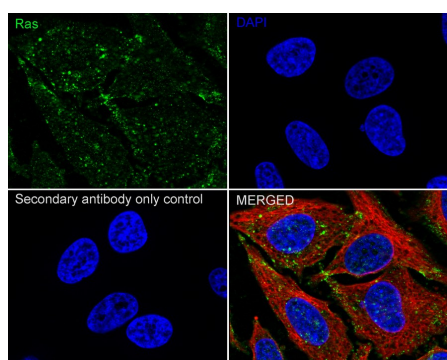
Observed band size: 21 kDa

Exposure time: 25 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

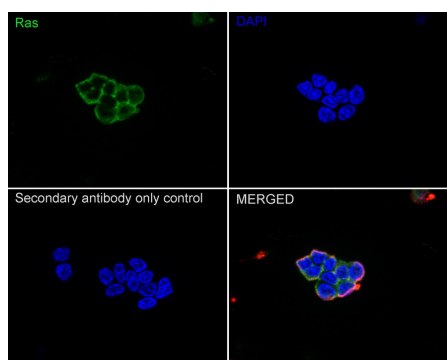
Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ER40115) at 1/1,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

**Fig2:** Immunocytochemistry analysis of HeLa cells labeling Ras with Rabbit anti-Ras antibody (ER40115) 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 (ER40115) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. 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.

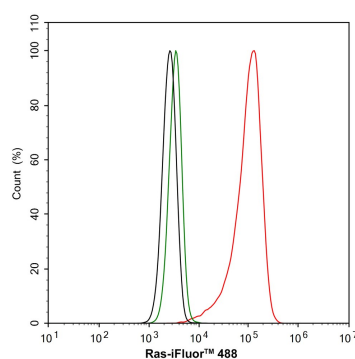
Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.



**Fig3:** Immunocytochemistry analysis of PC-12 cells labeling Ras with Rabbit anti-Ras antibody (ER40115) at 1/100 dilution.

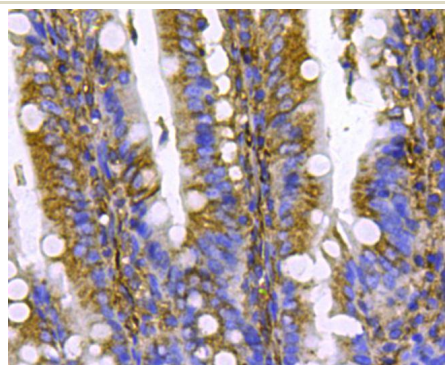
Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Ras antibody (ER40115) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. 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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

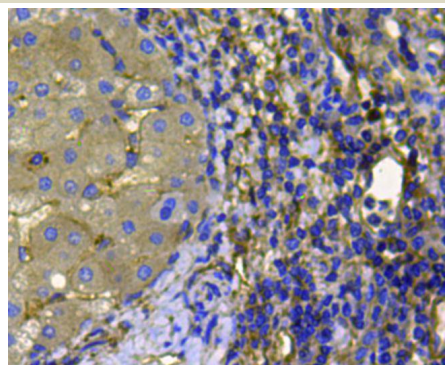


**Fig4:** Flow cytometric analysis of HeLa cells labeling Ras.

Cells were fixed and permeabilized. Then stained with the primary antibody (ER40115, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C 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°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).



**Fig5:** Immunohistochemical analysis of paraffin-embedded rat small intestine tissue using anti-Ras antibody. Counter stained with hematoxylin.



**Fig6:** Immunohistochemical analysis of paraffin-embedded human liver cancer tissue using anti-Ras antibody. Counter stained with hematoxylin.

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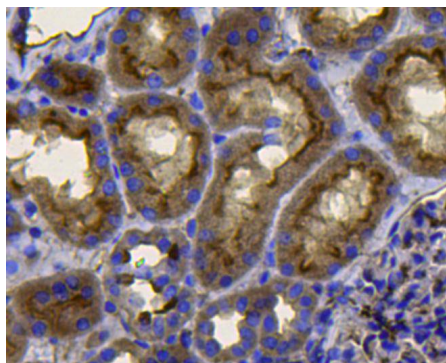
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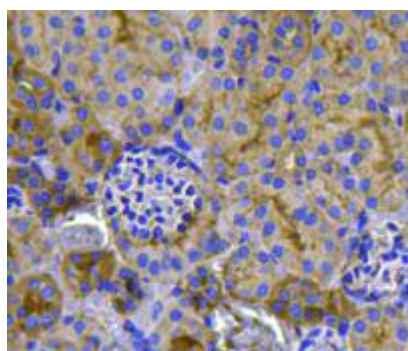
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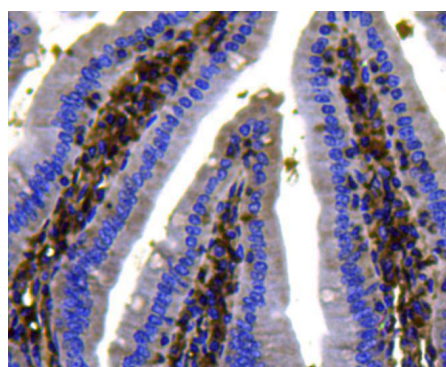
Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation



**Fig7:** Immunohistochemical analysis of paraffin-embedded human kidney muscle tissue using anti-Ras antibody. Counter stained with hematoxylin.



**Fig8:** Immunohistochemical analysis of paraffin-embedded mouse kidney tissue using anti-Ras antibody. Counter stained with hematoxylin.



**Fig9:** Immunohistochemical analysis of paraffin-embedded mouse small intestine tissue using anti-Ras antibody. Counter stained with hematoxylin.

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

### Background References

1. "DHHC9 and GCP16 constitute a human protein fatty acyltransferase with specificity for H- and N-Ras." Swarthout J.T., Lobo S., Farh L., Croke M.R., Greentree W.K., Deschenes R.J., Linder M.E. J. Biol. Chem. 280:31141-31148(2005)
2. "An acylation cycle regulates localization and activity of palmitoylated Ras isoforms." Rocks O., Peyker A., Kahms M., Verveer P.J., Koerner C., Lumbierres M., Kuhlmann J., Waldmann H., Wittinghofer A., Bastiaens P.I.H. Science 307:1746-1752(2005)
3. "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)

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