

Anti-RhoA Antibody [SN0612]

ET1611-10



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell
Molecular Wt:	Predicted band size: 22 kDa
Clone number:	SN0612

Description: Transforming protein RhoA, also known as Ras homolog family member A (RhoA), is a small GTPase protein in the Rho family of GTPases that in humans is encoded by the RHOA gene. While the effects of RhoA activity are not all well known, it is primarily associated with cytoskeleton regulation, mostly actin stress fibers formation and actomyosin contractility. It acts upon several effectors. Among them, ROCK1 (Rho-associated, coiled-coil containing protein kinase 1) and DIAPH1 (Diaphanous Homologue 1, a.k.a. hDia1, homologue to mDia1 in mouse, diaphanous in Drosophila) are the best described. RhoA, and the other Rho GTPases, are part of a larger family of related proteins known as the Ras superfamily, a family of proteins involved in the regulation and timing of cell division. RhoA is one of the oldest Rho GTPases, with homologues present in the genomes since 1.5 billion years. As a consequence, RhoA is somehow involved in many cellular processes which emerged throughout evolution. RhoA specifically is regarded as a prominent regulatory factor in other functions such as the regulation of cytoskeletal dynamics, transcription, cell cycle progression and cell transformation.

Immunogen: Synthetic peptide within Human aa 144-193 / 193.

Positive control: HUVEC cell lysate, THP-1 cell lysate, HeLa cell lysate, SiHa cell lysate, mouse brain tissue lysate, rat brain tissue lysate, human brain tissue lysate, PC-12, human liver cancer tissue, mouse brain tissue.

Subcellular location: Cell membrane, Cytoplasm, Cleavage furrow, Midbody, Cell projection.

Database links: SwissProt: P61586 Human | Q9QU10 Mouse | P61589 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
IHC-P	1:50-1:200
IF-Cell	1:100

Storage Buffer: 1*TBS (pH7.4), 0.05% 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: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn

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Images

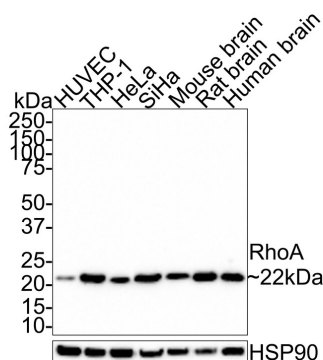


Fig1: Western blot analysis of RhoA on different lysates with Rabbit anti-RhoA antibody (ET1611-10) at 1/1,000 dilution.

Lane 1: HUVEC cell lysate (20 µg/Lane)
 Lane 2: THP-1 cell lysate (20 µg/Lane)
 Lane 3: HeLa cell lysate (20 µg/Lane)
 Lane 4: SiHa cell lysate (20 µg/Lane)
 Lane 5: Mouse brain tissue lysate (40 µg/Lane)
 Lane 6: Rat brain tissue lysate (40 µg/Lane)
 Lane 7: Human brain tissue lysate (40 µg/Lane)

Predicted band size: 22 kDa

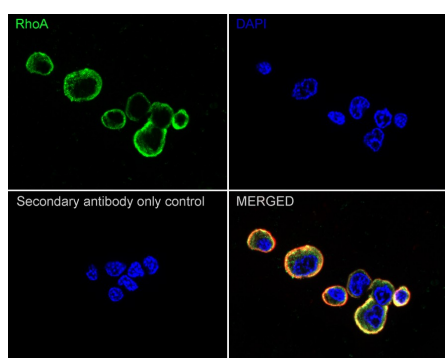
Observed band size: 22 kDa

Exposure time: 1 minute;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDN/TBST for 1 hour at room temperature. The primary antibody (ET1611-10) at 1/1,000 dilution was used in 5% NFDN/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

Fig2: Immunocytochemistry analysis of PC-12 cells labeling RhoA with Rabbit anti-RhoA antibody (ET1611-10) at 1/100 dilution.



Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-RhoA antibody (ET1611-10) 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 (M1305-2, 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.

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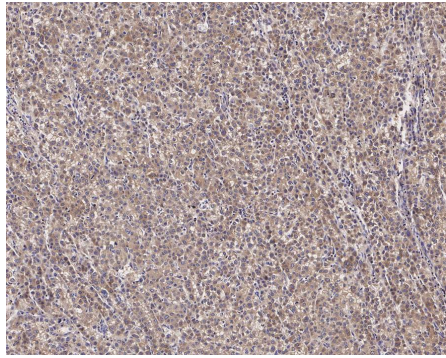


Fig3: Immunohistochemical analysis of paraffin-embedded human liver cancer tissue with Rabbit anti-RhoA antibody (ET1611-10) at 1/50 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (ET1611-10) at 1/50 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.

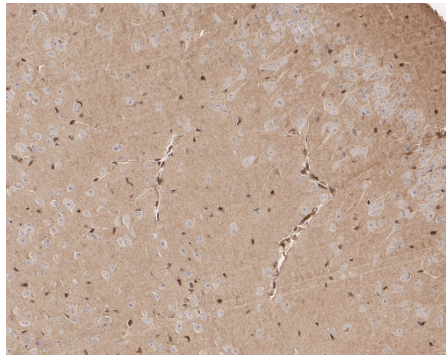


Fig4: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-RhoA antibody (ET1611-10) at 1/200 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (ET1611-10) at 1/200 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.

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

Background References

1. Freeman MC et al. Coronaviruses induce entry-independent, continuous macropinocytosis. *MBio* 5:e01340-14 (2014).
2. Nuno DW et al. RhoA localization with caveolin-1 regulates vascular contractions to serotonin. *Am J Physiol Regul Integr Comp Physiol* 303:R959-67 (2012).

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