Anti-FIP200 Antibody [A9A9]

HA601106

Product Type:	Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, FC
Molecular Wt:	Predicted band size: 183 kDa
Clone number:	A9A9

Description: FIP200 (FAK family kinase-interacting protein of 200 kDa) was identified in a two-hybrid screen with the tyrosine kinase Pyk2 and can inhibit Pyk2 kinase activity as well as related family members. FIP200 was later independently identified in a multi-drug resistance screen and named RB1CC1 (RB1-inducible coiled-coil 1) due to its induction by cytotoxic stress and RB1 expression regulation. FIP200 function has been linked to apoptosis, cell cycle progression, cell growth, and migration. FIP200 has also recently been shown to interact with ULK1 and is required for autophagosome formation. FIP200 is part of an ULK1 complex along with Atg13 that is regulated by mTOR and is required for starvation induced autophagy.

Immunogen: Recombinant protein within Human RB1CC1 aa 651-800 / 1,594.

Positive control: Hela cell lysate, MCF-7 cell lysate, 293T cell lysate, rat kidney tissue, human kidney tissue, Hela, MCF7, NIH/3T3.

Subcellular location: Nucleus, Cytoplasm, Cytoplasm, cytosol, Lysosome.

Database links: SwissProt: Q8TDY2 Human | Q9ESK9 Mouse
Entrez Gene: 312927 Rat

Recommended Dilutions:

WB	1:1,000
IHC-P	1:200-1:1,000
IF-Cell	1:200
FC	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: Immunogen affinity purified.

Images

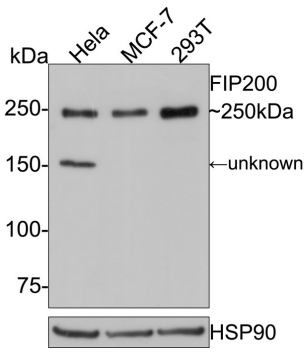

Fig1: Western blot analysis of FIP200 on different lysates with Mouse anti-FIP200 antibody (HA601106) at 1/1,000 dilution.

Lane 1: Hela cell lysate (30 µg/Lane)
Lane 2: MCF-7 cell lysate (30 µg/Lane)
Lane 3: 293T cell lysate (30 µg/Lane)

Predicted band size: 183 kDa
Observed band size: 250/150 kDa

Exposure time: 2 minutes;

6% 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 (HA601106) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:100,000 dilution was used for 1 hour at room temperature.

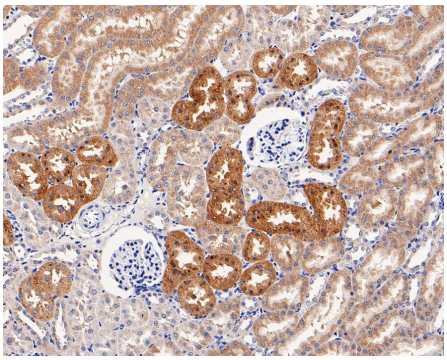

Fig2: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Mouse anti-FIP200 antibody (HA601106) at 1/1,000 dilution.

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 (HA601106) at 1/1,000 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.

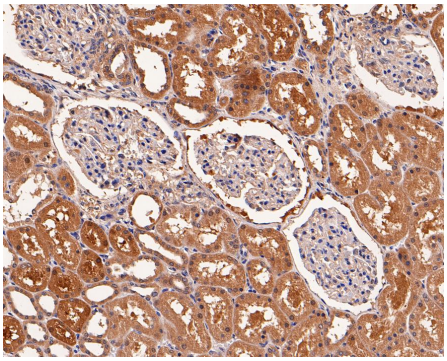

Fig3: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Mouse anti-FIP200 antibody (HA601106) at 1/200 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (HA601106) 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.

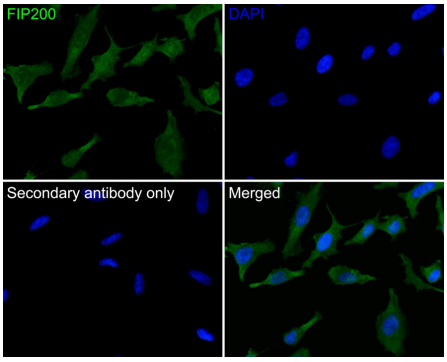

Fig4: Immunocytochemistry analysis of Hela cells labeling FIP200 with Mouse anti-FIP200 antibody (HA601106) at 1/200 dilution.

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-FIP200 antibody (HA601106) at 1/200 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.

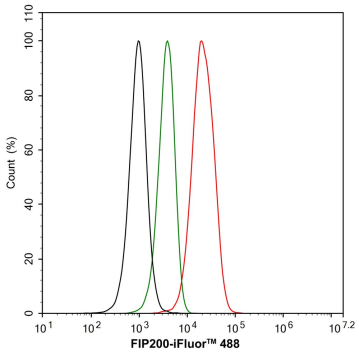

Fig5: Flow cytometric analysis of MCF7 cells labeling FIP200.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA601106, 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).

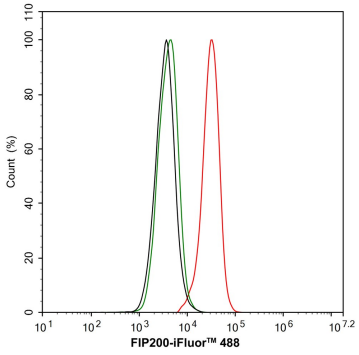

Fig6: Flow cytometric analysis of NIH/3T3 cells labeling FIP200.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA601106, 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).

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

Background References

1. Yeo SK et al. Role of FIP200 in inflammatory processes beyond its canonical autophagy function. Biochem Soc Trans. 2020 Aug
2. Wang L et al. FIP200 restricts RNA virus infection by facilitating RIG-I activation. Commun Biol. 2021 Jul