Anti-RAB7 Antibody [SN202-03] - BSA and Azide free

HA750279

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC
Molecular Wt:	Predicted band size: 23 kDa
Clone number:	SN202-03

Description: The Ras-related superfamily of guanine nucleotide binding proteins, which includes the Ral/Rec, Rap, R-Ras, and Rho/Rab subfamilies, exhibit 30-60% homology with Ras p21. Accumulating data suggests an important role for Rab proteins, either in endocytosis or in biosynthetic protein transport. The transport of newly synthesized proteins from the endoplasmic reticulum to various stacks of the Golgi complex and to secretory vesicles involves at each stage the movement of carrier vesicles, a process that appears to involve Rab protein function. The possibility that Rab proteins might also direct the exocytosis from secretory vesicles to the plasma membrane is supported by the observation that in yeast, the Sec4 protein, which is 40% homologous to Rab proteins, is associated with secretory vesicles. Several members of the Rab subfamily have been identified, each of which is found at a particular stage of a membrane transport pathway.

Immunogen: Synthetic peptide within Human RAB7 aa 158-207 / 207.

Positive control: HeLa cell lysate, MCF7 cell lysate, Neuro-2a cell lysate, C2C12 cell lysate, PC-12 cell lysate, C6 cell lysate, Hela, SW480, NIH/3T3, human colon carcinoma tissue, human kidney tissue, mouse skeletal tissue, mouse kidney tissue, K562.

Subcellular location: Cytoplasmic vesicle, Lysosome membrane, Melanosome membrane, Lipid droplet.

Database links: SwissProt: P51149 Human | P51150 Mouse | P09527 Rat

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:100-1:1,000
IF-Tissue	1:100-1:500
IHC-P	1:50-1:1,000
FC	1:500-1:1,000

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

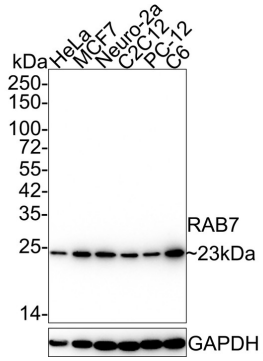

Fig1: Western blot analysis of RAB7 on different lysates with Rabbit anti-RAB7 antibody (HA750279) at 1/5,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: MCF7 cell lysate
Lane 3: Neuro-2a cell lysate
Lane 4: C2C12 cell lysate
Lane 5: PC-12 cell lysate
Lane 6: C6 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 23 kDa
Observed band size: 23 kDa

Exposure time: 17 seconds;

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 (HA750279) at 1/5,000 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.

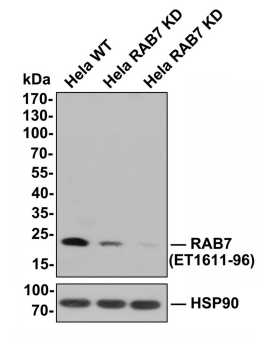

Fig2: All lanes: Western blot analysis of RAB7 with anti-RAB7 antibody (HA750279) at 1:500 dilution.
Lane 1: Wild-type Hela whole cell lysate (10 µg).
Lane 2/3: RAB7 knockdown Hela whole cell lysate (10 µg).

ET1611-96 was shown to specifically react with RAB7 in wild-type Hela cells. Weakened bands were observed when RAB7 knockdown samples were tested. Wild-type and RAB7 knockdown samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1611-96, 1:500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG-HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

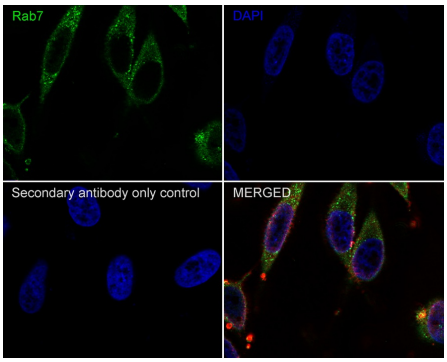

Fig3: Immunocytochemistry analysis of Hela cells labeling RAB7 with Rabbit anti-RAB7 antibody (HA750279) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-RAB7 antibody (HA750279) at 1/200 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, 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.

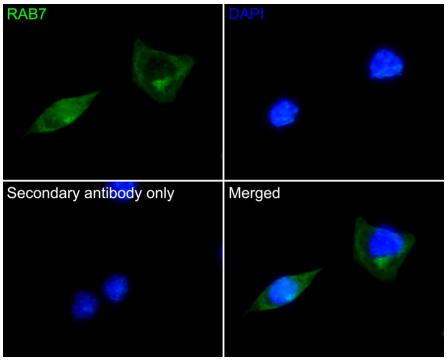

Fig4: Immunocytochemistry analysis of SW480 cells labeling RAB7 with Rabbit anti-RAB7 antibody (HA750279) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-RAB7 antibody (HA750279) at 1/50 dilution in 2% negative goat serum 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.

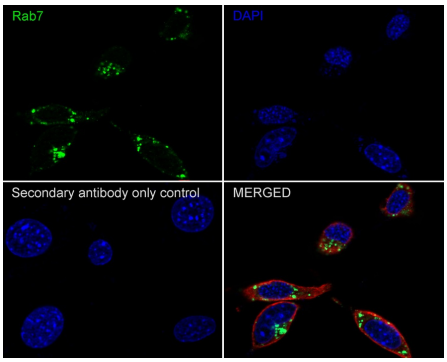

Fig5: Immunocytochemistry analysis of NIH/3T3 cells labeling RAB7 with Rabbit anti-RAB7 antibody (HA750279) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-RAB7 antibody (HA750279) at 1/200 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, 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.

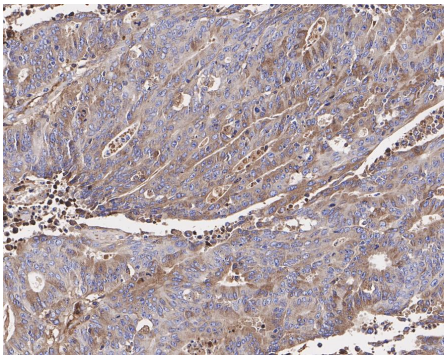

Fig6: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Rabbit anti-RAB7 antibody (HA750279) at 1/1,000 dilution.

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 (HA750279) 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.

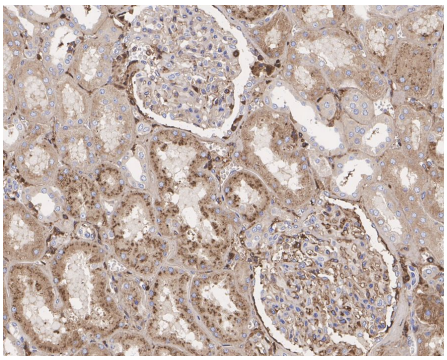

Fig7: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-RAB7 antibody (HA750279) at 1/1,000 dilution.

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 (HA750279) 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.

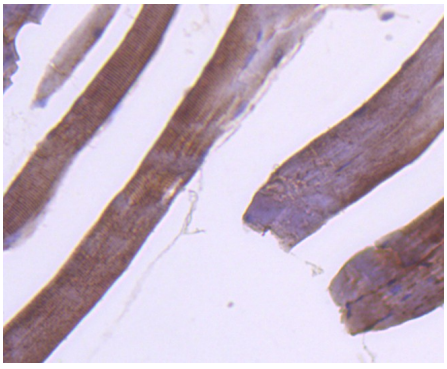

Fig8: Immunohistochemical analysis of paraffin-embedded mouse skeletal tissue using anti-RAB7 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750279, 1/50) for 30 minutes 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.

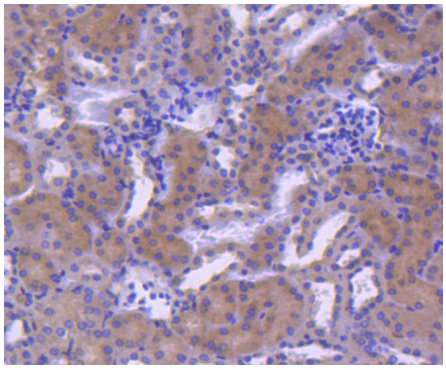

Fig9: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue using anti-RAB7 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750279, 1/50) for 30 minutes 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.

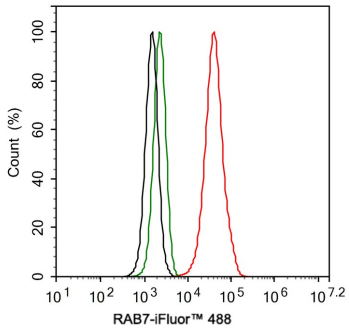

Fig10: Flow cytometric analysis of RAB7 was done on K562 cells. The cells were fixed, permeabilized and stained with the primary antibody (HA750279, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

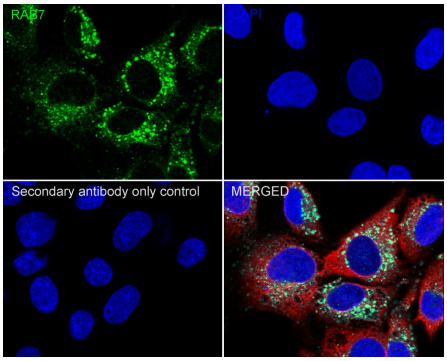

Fig11: Immunocytochemistry analysis of C6 cells labeling RAB7 with Rabbit anti-RAB7 antibody (HA750279) at 1/1,000 dilution.

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-RAB7 antibody (HA750279) at 1/1,000 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 (M1305-2, 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.

Background References

1. Fields J et al. HIV-1 Tat alters neuronal autophagy by modulating autophagosome fusion to the lysosome: implications for HIV-associated neurocognitive disorders. J Neurosci 35:1921-38 (2015).
2. Kanagaraj P et al. Souffle/Spastizin Controls Secretory Vesicle Maturation during Zebrafish Oogenesis. PLoS Genet 10:e1004449 (2014).