LRP1-mediated Endocytosis and Transmission of Tau Antibody Sampler Kit

HAK21098

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
Hsp70 [ET1601-11]	20µl	WB,IF-Cell,IF-Tissue,IHC-P,FC	H,M,R	70 kDa
Apolipoprotein E [ET1610-22]	20µl	WB,IF-Cell,IF-Tissue,IHC-P,IP	H,M	36 kDa
Tau [ET1603-2]	20µl	WB,IHC-P,IP,IHC-Fr	H,M,R	79 kDa
Phospho-Tau (T181) [HA722271]	20µl	WB,IHC-P	H,M	79 kDa
Phospho-Tau (S404) [HA721801]	20µl	WB,IHC-P	H,M,R	79 kDa
SorLA [HA721337]	20µl	WB,IHC-P	H,M,R	248 kDa
Rab5 [ET1609-27]	20µl	WB,IHC-P,IF-Cell	H,M,R	24 kDa
RAB7 [ET1611-96]	20µl	WB,IF-Cell,IF-Tissue,IHC-P,FC	H,M,R	23 kDa
Rab11A [HA721552]	20µl	WB,IHC-P	H,M,R	24 kDa
HRP-Goat anti-Rabbit IgG [HA1001]	100µl	WB,ELISA,IHC-P	Rab	

Description: The LRP1-mediated Endocytosis and Transmission of Tau Antibody Sampler Kit provides an economical means of detecting components of the LRP-1 mediated intercellular transmission of human tau using antibodies. The kit includes enough antibodies to perform two western blot experiments with each primary antibody.

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

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

Background Tau is a heterogeneous microtubule-associated protein that promotes and stabilizes microtubule assembly, especially in axons. In addition to its normal function, intracellular neurofibrillary tangle protein aggregates, composed of hyperphosphorylated helical bundles of tau, are a major hallmark of neurodegenerative diseases like Alzheimer's disease (AD). Moreover, disease progression is also measured by the progressive spread and deposition of the protein aggregates via intercellular transfer of tau. Although the intercellular mechanism of protein aggregate transfer is poorly understood, low density lipoprotein receptor related protein 1 (LRP1) was identified as a regulator of tau uptake and spread. LRP1 is a type I transmembrane receptor that mediates the endocytosis of various ligands, including apolipoproteins and tau. Interestingly, human apolipoprotein E (ApoE), which also binds to LRP1, is genetically linked to AD. LRP1-mediated protein uptake, in addition to tau, may play an important role in AD progression. In addition to LRP1, other low density lipoprotein receptor related proteins, including SORL1, are genetically linked to AD, suggesting a conserved cellular mechanism that converges on this family of proteins that contributes to AD etiology. Once tau binds to LRP1, receiving cells are likely to internalize and process tau via the endosomal pathway, completing cell-to-cell transmission. Rab5, Rab7, and Rab11, members of the Ras superfamily of small Rab GTPases, are likely to regulate endosomal processing of tau.

Database links: UniProt ID: P17879, Q61696, P0DMV9, P0DMV8, Q07439, P02649, P08226, P10636,

Images

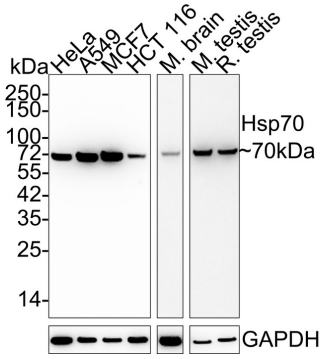

Fig1: Western blot analysis of Hsp70 on different lysates with Rabbit anti-Hsp70 antibody (ET1601-11) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: A549 cell lysate
Lane 3: MCF7 cell lysate
Lane 4: HCT 116 cell lysate
Lane 5: Mouse brain tissue lysate
Lane 6: Mouse testis tissue lysate
Lane 7: Rat testis tissue lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 70 kDa
Observed band size: 70 kDa

Exposure time: Lane 1-4: 43 seconds; Lane 5-7: 2 minutes;

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 (ET1601-11) at 1/2,000 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.

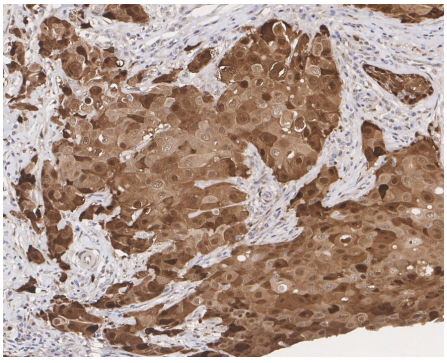

Fig2: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue with Rabbit anti-Hsp70 antibody (ET1601-11) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (ET1601-11) 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.

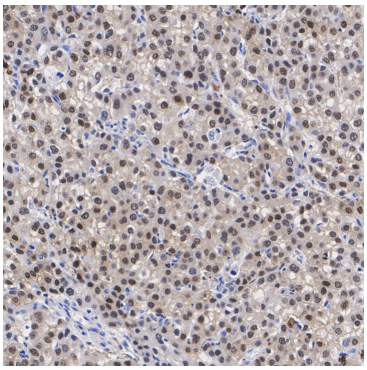

Fig3: Immunohistochemical analysis of paraffin-embedded human liver cancer tissue with Rabbit anti-Hsp70 antibody (ET1601-11) at 1/500 dilution.

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 (ET1601-11) at 1/500 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.

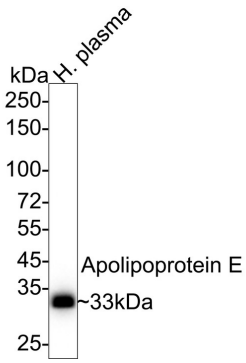

Fig4: Western blot analysis of Apolipoprotein E on human plasma tissue lysates with Rabbit anti-Apolipoprotein E antibody (ET1610-22) at 1/1,000 dilution.

Lysates/proteins at 20 µg/Lane.

Predicted band size: 36 kDa
Observed band size: 33 kDa

Exposure time: 6 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 (ET1610-22) 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.

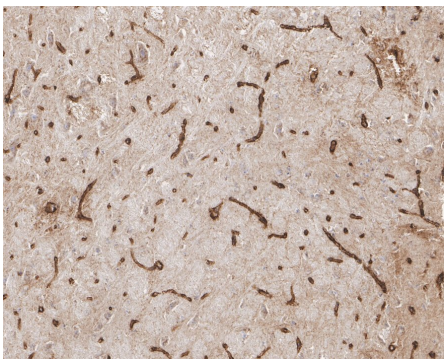

Fig5: Immunohistochemical analysis of paraffin-embedded AD mouse brain tissue with Rabbit anti-Apolipoprotein E antibody (ET1610-22) at 1/1,000 dilution.

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 (ET1610-22) 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.

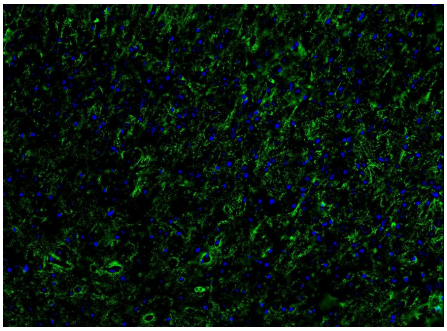

Fig6: Immunofluorescence analysis of frozen mouse cerebral cortex tissue labeling Tau with Rabbit anti-Tau antibody (ET1603-2).

The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1603-2, green) at 1/50 dilution overnight at 4℃, washed with PBS. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue). Image acquisition was performed with KFBIO KF-FL-400 Scanner.

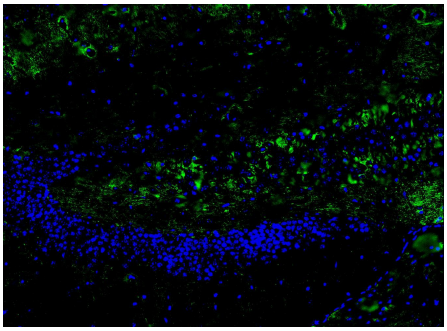

Fig7: Immunofluorescence analysis of frozen mouse hippocampus tissue labeling Tau with Rabbit anti-Tau antibody (ET1603-2).

The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1603-2, green) at 1/50 dilution overnight at 4℃, washed with PBS. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue). Image acquisition was performed with KFBIO KF-FL-400 Scanner.

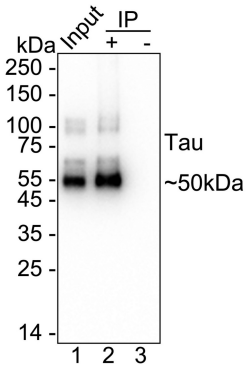

Fig8: Tau was immunoprecipitated from 0.2 mg mouse brain tissue lysate with ET1603-2 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using ET1603-2 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: Mouse brain tissue lysate (input)
Lane 2: ET1603-2 IP in mouse brain tissue lysate
Lane 3: Rabbit IgG instead of ET1603-2 in mouse brain tissue lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 3 seconds; ECL: K1801

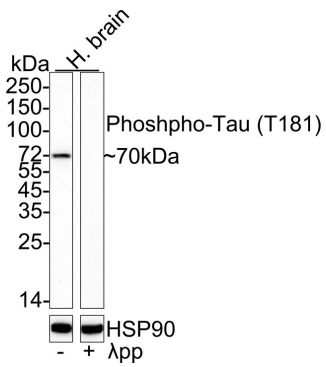

Fig9: Western blot analysis of Phospho-Tau (T181) on different lysates with Rabbit anti-Phospho-Tau (T181) antibody (HA722271) at 1/1,000 dilution.

Lane 1: Human brain tissue lysate
Lane 2: Human brain tissue lysate, the membrane treated with λpp for 1 hour
Lysates/proteins at 40 µg/Lane.

Predicted band size: 79 kDa
Observed band size: 70 kDa

Exposure time: 3 minutes; 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 (HA722271) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

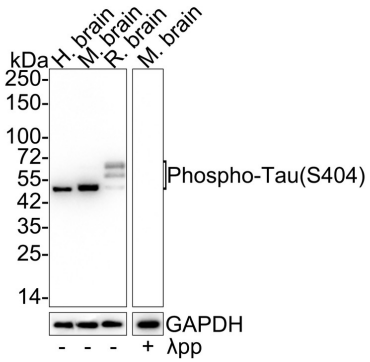

Fig10: Western blot analysis of Phospho-Tau (S404) on different lysates with Rabbit anti-Phospho-Tau (S404) antibody (HA721801) at 1/1,000 dilution.

Lane 1: Human brain tissue lysate
Lane 2: Mouse brain tissue lysate
Lane 3: Rat brain tissue lysate
Lane 4: Mouse brain tissue lysate, the membrane treated with λpp for 1 hour

Lysates/proteins at 30 µg/Lane.

Predicted band size: 79 kDa
Observed band size: 50-70 kDa

Exposure time: 1 minute;

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 (HA721801) 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.

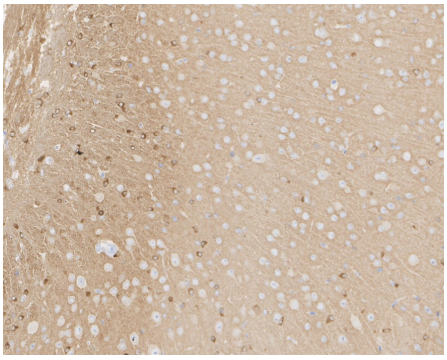

Fig11: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-Phospho-Tau (S404) antibody (HA721801) at 1/1,000 dilution.

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 (HA721801) 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.

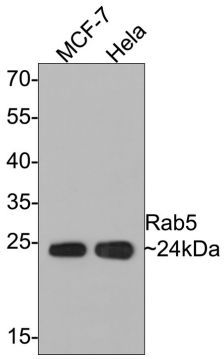

Fig12: Western blot analysis of Rab5 on different lysates with Rabbit anti-Rab5 antibody (ET1609-27) at 1/1,000 dilution.

Lane 1: MCF-7 cell lysate
Lane 2: Hela cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 24 kDa
Observed band size: 24 kDa

Exposure time: 1 minute;

12% 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 (ET1609-27) at 1/1,000 dilution was used in 5% NFDM/TBST 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.

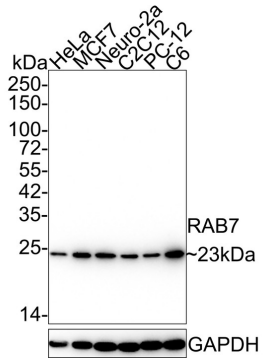

Fig13: Western blot analysis of RAB7 on different lysates with Rabbit anti-RAB7 antibody (ET1611-96) at 1/5,000 dilution.

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 (ET1611-96) 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.

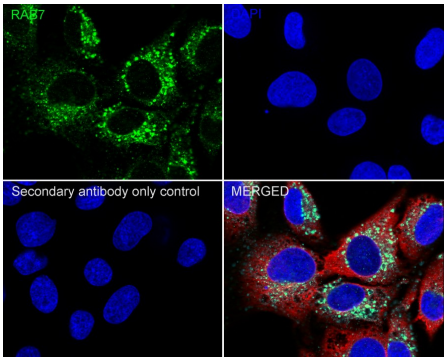

Fig14: Immunocytochemistry analysis of C6 cells labeling RAB7 with Rabbit anti-RAB7 antibody (ET1611-96) at 1/1,000 dilution.

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 (ET1611-96) 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.

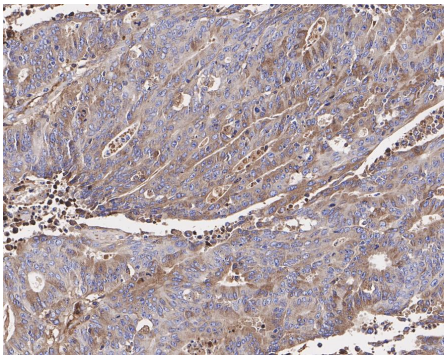

Fig15: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Rabbit anti-RAB7 antibody (ET1611-96) at 1/1,000 dilution.

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 (ET1611-96) 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.

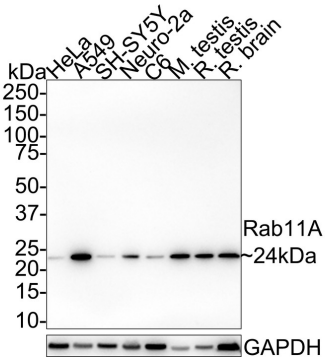

Fig16: Western blot analysis of Rab11A on different lysates with Rabbit anti-Rab11A antibody (HA721552) at 1/1,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: A549 cell lysate (20 µg/Lane)
Lane 3: SH-SY5Y cell lysate (20 µg/Lane)
Lane 4: Neuro-2a cell lysate (20 µg/Lane)
Lane 5: C6 cell lysate (20 µg/Lane)
Lane 6: Mouse testis tissue lysate (40 µg/Lane)
Lane 7: Rat testis tissue lysate (40 µg/Lane)
Lane 8: Rat brain tissue lysate (40 µg/Lane)

Predicted band size: 24 kDa
Observed band size: 24 kDa

Exposure time: 2 minutes 37 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 (HA721552) at 1/1,000 dilution was used in 5% NFDM/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.

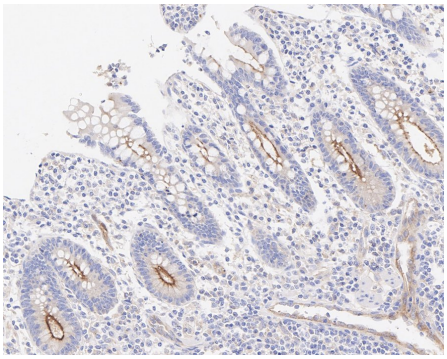

Fig17: Immunohistochemical analysis of paraffin-embedded human small intestine tissue with Rabbit anti-Rab11A antibody (HA721552) at 1/1,000 dilution.

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 (HA721552) 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.

Background References

1. Johnson, G.V. and Stoothoff, W.H. (2004) J Cell Sci 117, 5721-9.
2. Braak, H. and Braak, E. (1991) Acta Neuropathol 82, 239-59.
3. Rauch, J.N. et al. (2020) Nature 580, 381-385.
4. Corder, E.H. et al. (1993) Science 261, 921-3.
5. Yin, R.H. et al. (2015) Mol Neurobiol 51, 909-18.
6. Zhang, X. et al. (2019) ACS Chem Neurosci 10, 828-838.