PICALM Signaling Antibody Sampler Kit

HAK21026

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
PICALM [HA721706]	20µl	WB,IHC-P	H,M,R	71 kDa
Clathrin heavy chain [ET1704-50]	20µl	WB,IHC-P	H,M,R,Mk	180kDa
LAMP1 [ET1701-94]	20µl	WB,IHC-P,IF-Tissue	H	45 kDa
EEA1 [HA722147]	20µl	WB,IF-Cell,FC	H,M,R,Mk	162 kDa
Cathepsin D [ET1608-49]	20µl	WB,IF-Cell,IHC-P,IP	H,M,R	45/28 kDa
HRP-Goat Anti-Rabbit IgG (H+L) [HA1001]	100µl	WB,ELISA,IHC-P	Rab	

Description: The PICALM Signaling Antibody Sampler Kit provides an economical means of investigating PICALM signaling by western blot and labeling endo-lysosomal components by immunofluorescence (IF). This kit includes enough primary antibodies to perform two western blot experiments or at least 40 IF tests per primary antibody.

Storage Buffer: 1*TBS (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 The antibodies in this kit serve to characterize phosphatidylinositol-binding clathrin assembly protein (PICALM)-mediated lysosomal maturation, as endo-lysosomal systems are important for normal physiology and prevention of common late-onset neurodegenerative diseases such as Alzheimer's disease (AD). PICALM is a clathrin-binding protein involved in the endo-lysosomal pathway, where it has been genetically associated with AD .

PICALM disruption increases the number of early endosomes, which is linked to exacerbated tau aggregation. Early endosome antigen 1 (EEA1) is an early endosomal marker and a Rab5 effector protein essential for early endosomal membrane fusion and trafficking . Lysosome-associated membrane protein 1 (LAMP1) is an abundant lysosomal membrane protein involved in regulating lysosomal motility during lysosome-phagosome fusion . Cathepsin D (CSTD) is a ubiquitously expressed lysosomal aspartyl protease involved in the normal degradation of proteins. Mutations in PICALM were shown to cause lysosomal enzymes and membrane proteins to be mis-trafficked and accumulated.

Database links: UniProt ID: Q13492, Q7M6Y3, O55012, Q00610, Q68FD5, P11442, P11279, Q15075, Q8BL66, 314764, P07339, P18242, P24268

Images

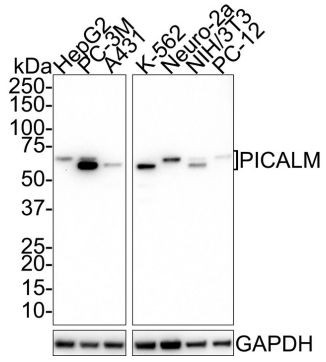

Fig1: Western blot analysis of PICALM on different lysates with Rabbit anti-PICALM antibody (HA721706) at 1/1,000 dilution.

Lane 1: HepG2 cell lysate
Lane 2: PC-3M cell lysate
Lane 3: A431 cell lysate
Lane 4: K-562 cell lysate
Lane 5: Neuro-2a cell lysate
Lane 6: NIH/3T3 cell lysate
Lane 7: PC-12 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 71 kDa
Observed band size: 65/71 kDa

Exposure time: 3 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 (HA721706) 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.

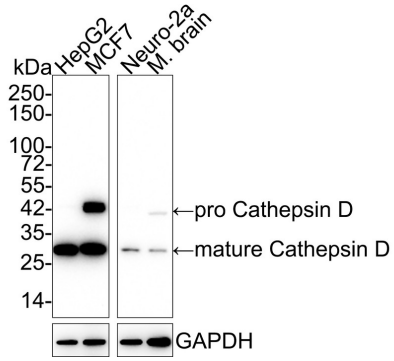

Fig2: Western blot analysis of Cathepsin D on different lysates with Rabbit anti-Cathepsin D antibody (ET1608-49) at 1/2,000 dilution.

Lane 1: HepG2 cell lysate (15 µg/Lane)
Lane 2: MCF7 cell lysate (15 µg/Lane)
Lane 3: Neuro-2a cell lysate (15 µg/Lane)
Lane 4: Mouse brain tissue lysate (20 µg/Lane)

Predicted band size: 45/28 kDa
Observed band size: 45/28 kDa

Exposure time: 3 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 (ET1608-49) 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.

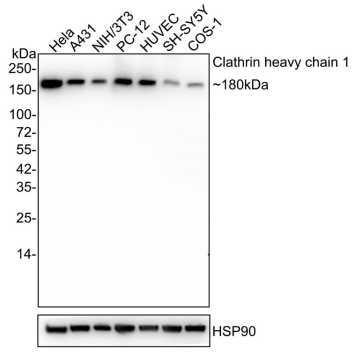

Fig3: Western blot analysis of Clathrin heavy chain on different lysates with Rabbit anti-Clathrin heavy chain antibody (ET1704-50) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: A431 cell lysate
Lane 3: NIH/3T3 cell lysate
Lane 4: PC-12 cell lysate
Lane 5: HUVEC cell lysate
Lane 6: SH-SY5Y cell lysate
Lane 7: COS-1 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 180kDa
Observed band size: 180 kDa

Exposure time: 10 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 (ET1704-50) 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.

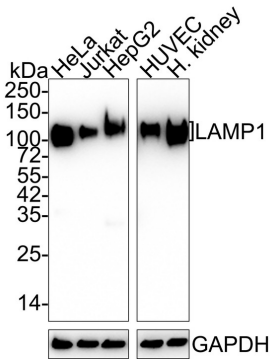

Fig4: Western blot analysis of LAMP1 on different lysates with Rabbit anti-LAMP1 antibody (ET1701-94) at 1/1,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: Jurkat cell lysate (20 µg/Lane)
Lane 3: HepG2 cell lysate (20 µg/Lane)
Lane 4: HUVEC cell lysate (20 µg/Lane)
Lane 5: Human kidney tissue lysate (40 µg/Lane)

Predicted band size: 45 kDa
Observed band size: 100-120 kDa

Exposure time: 1 minute 2 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 (ET1701-94) 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.

Background References

1. Kaksonen, M. and Roux, A. (2018) Nat Rev Mol Cell Biol 19, 313-326.
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3. Mu, F.T. et al. (1995) J Biol Chem 270, 13503-11.
4. Christoforidis, S. et al. (1999) Nature 397, 621-5.
5. Eskelinen, E.L. et al. (2003) Trends Cell Biol 13, 137-45.
6. Huynh, K.K. et al. (2007) EMBO J 26, 313-24.
7. Faust, P.L. et al. (1985) Proc Natl Acad Sci USA 82, 4910-4.