γ Secretase Antibody Sampler Kit

HAK21087

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
Nicastrin [ET7108-38]	20μl	WB	H,M	78 kDa
PEN2 [ET7109-26]	20μl	WB,IF-Cell,IHC-P,FC,IHC-Fr,IF-Tissue	H,M,R	12 kDa
Presenilin 1 [HA722995]	20μl	IHC-P,IHC-Fr,IF-Tissue	H,M,R	53 kDa
Presenilin 2/AD5 [HA722243]	20μl	WB,IP	H,M,R	50 kDa
HRP-Goat Anti-Rabbit IgG (H+L) [HA1001]	100μl	WB,ELISA,IHC-P	Rab	

Description: The γ Secretase Antibody Sampler Kit provides an economical means of evaluating components of the gamma secretase complex. The kit contains enough primary and secondary antibodies to perform two western miniblot experiments.

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 γ secretase protease complex interacts with and cleaves intramembrane substrates as an essential function for regulation of intracellular signaling and cell-cell interactions. This multiprotein complex is comprised of four integral membrane proteins, Presenilin, Nicastrin, Aph-1, and PEN2, all of which are essential for complete proteolytic activity.

Presenilin 1 and presenilin 2 are transmembrane proteins belonging to the presenilin family. Nicastrin is a transmembrane glycoprotein serving as an essential component of the γ-secretase complex. Nicastrin protein is physically associated with presenilin and plays an important role in stabilization and correct localization of presenilin to the membrane-bound γ-secretase complex. Nicastrin also serves as a docking site for γ-secretase substrates such as APP and Notch, directly binding to them and presenting them properly to γ-secretase to ensure the correct cleavage process. Presenilin Enhancer 2 (PEN2) is an important part of the γ-secretase complex as its knock down results in reduced amounts of the complex, resulting in a loss of γ-secretase activity.

Database links: UniProt ID: Q92542, P57716, Q9NZ42, Q9CQR7, O88777, P49768, P49769, P97887, P49810, Q61144, O88777

Images

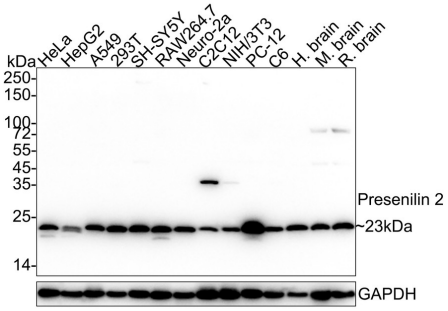

Fig1: Western blot analysis of Presenilin 2/AD5 on different lysates with Rabbit anti-Presenilin 2/AD5 antibody (HA722243) at 1/1,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: HepG2 cell lysate (20 µg/Lane)
Lane 3: A549 cell lysate (20 µg/Lane)
Lane 4: 293T cell lysate (20 µg/Lane)
Lane 5: SH-SY5Y cell lysate (20 µg/Lane)
Lane 6: RAW264.7 cell lysate (20 µg/Lane)
Lane 7: Neuro-2a cell lysate (20 µg/Lane)
Lane 8: C2C12 cell lysate (20 µg/Lane)
Lane 9: NIH/3T3 cell lysate (20 µg/Lane)
Lane 10: PC-12 cell lysate (20 µg/Lane)
Lane 11: C6 cell lysate (20 µg/Lane)
Lane 12: Human brain tissue lysate (40 µg/Lane)
Lane 13: Mouse brain tissue lysate (40 µg/Lane)
Lane 14: Rat liver tissue lysate (40 µg/Lane)

Predicted band size: 50 kDa
Observed band size: 23 kDa

Exposure time: 1 minute; 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 (HA722243) 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.

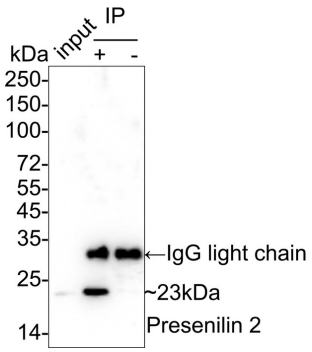

Fig2: Presenilin 2/AD5 was immunoprecipitated from 0.2 mg HeLa cell lysate with HA722243 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using HA722243 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: HA722243 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of HA722243 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 59 seconds; ECL: K1801

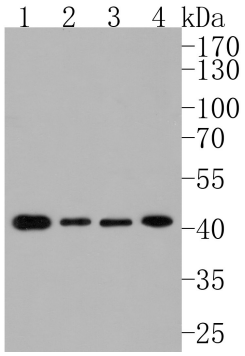

Fig3: Western blot analysis of Presenilin 1/PS-1 on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (HA500219, 1/1,000) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

Positive control:

Lane 1: NIH/3T3 cell lysate
Lane 2: Raji cell lysate
Lane 3: MCF-7 cell lysate
Lane 4: Daudi cell lysate

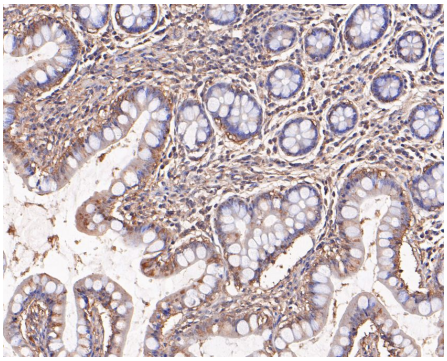

Fig4: Immunohistochemical analysis of paraffin-embedded human small intestine tissue using anti-Presenilin 1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA500219, 1/400) 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.

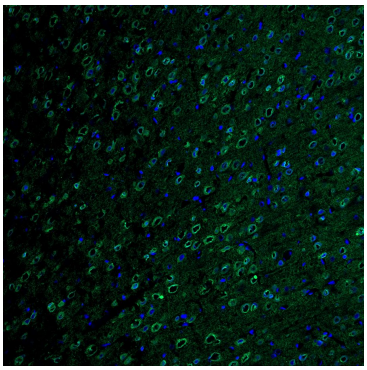

Fig5: Immunofluorescence analysis of frozen rat brain tissue with Rabbit anti-PEN2 antibody (ET7109-26) at 1/100 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET7109-26, green) at 1/100 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

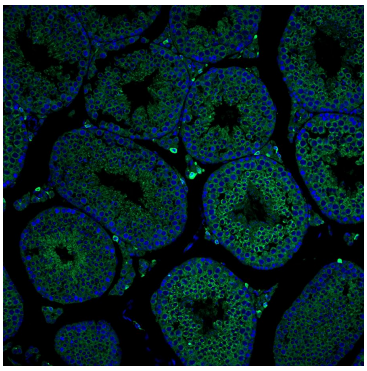

Fig6: Immunofluorescence analysis of paraffin-embedded mouse testis tissue labeling PEN2 with Rabbit anti-PEN2 antibody (ET7109-26) at 1/200 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET7109-26, green) at 1/200 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

Background References

1. Sala Frigerio C, Piscopo P, Calabrese E, Crestini A, Malvezzi Campeggi L, Civita di Fava R, Fogliarino S, Albani D, Marcon G, Cherchi R, Piras R, Forloni G, Confaloni A. PEN-2 gene mutation in a familial Alzheimer's disease case. J Neurol. 2005 Sep;252(9):1033-6.
2. Hansson CA, Frykman S, Farmery MR, Tjernberg LO, Nilsberth C, Pursglove SE, Ito A, Winblad B, Cowburn RF, Thyberg J, Ankarcrona M. Nicastrin, presenilin, APH-1, and PEN-2 form active gamma-secretase complexes in mitochondria. J Biol Chem. 2004 Dec 3;279(49):51654-60.
3. Esler WP, Kimberly WT, Ostaszewski BL, Ye W, Diehl TS, Selkoe DJ, Wolfe MS. Activity-dependent isolation of the presenilin- gamma -secretase complex reveals nicastrin and a gamma substrate. Proc Natl Acad Sci U S A. 2002 Mar 5;99(5):2720-5.