abcam

Product datasheet

Anti-Amyloid Precursor Protein antibody [EPR5119(2)] ab133588

KO VALIDATED Recombinant RabMAb

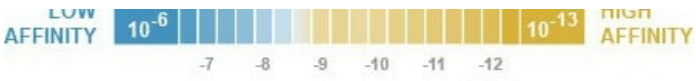
2 References 8 Images

Overview

Product name	Anti-Amyloid Precursor Protein antibody [EPR5119(2)]
Description	Rabbit monoclonal [EPR5119(2)] to Amyloid Precursor Protein
Host species	Rabbit
Tested applications	**Suitable for:** WB, IP **Unsuitable for:** ICC/IF or IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human Amyloid Precursor Protein aa 650-750. The exact sequence is proprietary. Database link: **P05067**
Positive control	293T, SH-SY5Y, U87 MG and Human fetal brain lysates
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Dissociation constant (K_D)	$K_D = 9.50 \times 10^{-11}$ M



Learn more about K_D

Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR5119(2)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab133588 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/2000. Detects a band of approximately 100-120 kDa (predicted molecular weight: 86 kDa).
IP		1/30.

Application notes Is unsuitable for ICC/IF or IHC-P.

Target

Function

Functions as a cell surface receptor and performs physiological functions on the surface of neurons relevant to neurite growth, neuronal adhesion and axonogenesis. Involved in cell mobility and transcription regulation through protein-protein interactions. Can promote transcription activation through binding to APBB1-KAT5 and inhibits Notch signaling through interaction with Numb. Couples to apoptosis-inducing pathways such as those mediated by G(O) and JIP. Inhibits G(o) alpha ATPase activity (By similarity). Acts as a kinesin I membrane receptor, mediating the axonal transport of beta-secretase and presenilin 1. Involved in copper homeostasis/oxidative stress through copper ion reduction. In vitro, copper-metallated APP induces neuronal death directly or is potentiated through Cu(2+)-mediated low-density lipoprotein oxidation. Can regulate neurite outgrowth through binding to components of the extracellular matrix such as heparin and collagen I and IV. The splice isoforms that contain the BPTI domain possess protease inhibitor activity. Induces a AGER-dependent pathway that involves activation of p38 MAPK, resulting in internalization of amyloid-beta peptide and leading to mitochondrial dysfunction in cultured cortical neurons. Provides Cu(2+) ions for GPC1 which are required for release of nitric oxide (NO) and subsequent degradation of the heparan sulfate chains on GPC1.
Beta-amyloid peptides are lipophilic metal chelators with metal-reducing activity. Bind transient metals such as copper, zinc and iron. In vitro, can reduce Cu(2+) and Fe(3+) to Cu(+) and Fe(2+), respectively. Beta-amyloid 42 is a more effective reductant than beta-amyloid 40. Beta-amyloid peptides bind to lipoproteins and apolipoproteins E and J in the CSF and to HDL particles in plasma, inhibiting metal-catalyzed oxidation of lipoproteins. Beta-APP42 may activate mononuclear phagocytes in the brain and elicit inflammatory responses. Promotes both tau

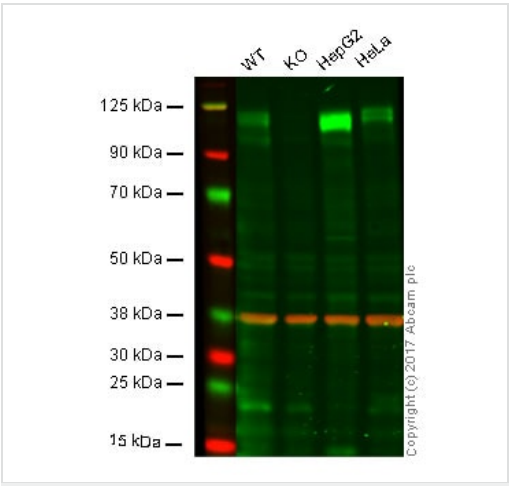
	aggregation and TPK II-mediated phosphorylation. Interaction with overexpressed HADH2 leads to oxidative stress and neurotoxicity. Also binds GPC1 in lipid rafts. Appicans elicit adhesion of neural cells to the extracellular matrix and may regulate neurite outgrowth in the brain. The gamma-CTF peptides as well as the caspase-cleaved peptides, including C31, are potent enhancers of neuronal apoptosis. N-APP binds TNFRSF21 triggering caspase activation and degeneration of both neuronal cell bodies (via caspase-3) and axons (via caspase-6).
Tissue specificity	Expressed in all fetal tissues examined with highest levels in brain, kidney, heart and spleen. Weak expression in liver. In adult brain, highest expression found in the frontal lobe of the cortex and in the anterior perisylvian cortex-opercular gyri. Moderate expression in the cerebellar cortex, the posterior perisylvian cortex-opercular gyri and the temporal associated cortex. Weak expression found in the striate, extra-striate and motor cortices. Expressed in cerebrospinal fluid, and plasma. Isoform APP695 is the predominant form in neuronal tissue, isoform APP751 and isoform APP770 are widely expressed in non-neuronal cells. Isoform APP751 is the most abundant form in T-lymphocytes. Appican is expressed in astrocytes.
Involvement in disease	Alzheimer disease 1 Cerebral amyloid angiopathy, APP-related
Sequence similarities	Belongs to the APP family. Contains 1 BPTI/Kunitz inhibitor domain.
Domain	The basolateral sorting signal (BaSS) is required for sorting of membrane proteins to the basolateral surface of epithelial cells. The NPXY sequence motif found in many tyrosine-phosphorylated proteins is required for the specific binding of the PID domain. However, additional amino acids either N- or C-terminal to the NPXY motif are often required for complete interaction. The PID domain-containing proteins which bind APP require the YENPTY motif for full interaction. These interactions are independent of phosphorylation on the terminal tyrosine residue. The NPXY site is also involved in clathrin-mediated endocytosis.
Post-translational modifications	Proteolytically processed under normal cellular conditions. Cleavage either by alpha-secretase, beta-secretase or theta-secretase leads to generation and extracellular release of soluble APP peptides, S-APP-alpha and S-APP-beta, and the retention of corresponding membrane-anchored C-terminal fragments, C80, C83 and C99. Subsequent processing of C80 and C83 by gamma-secretase yields P3 peptides. This is the major secretory pathway and is non-amyloidogenic. Alternatively, presenilin/nicastrin-mediated gamma-secretase processing of C99 releases the amyloid beta proteins, amyloid-beta 40 (Abeta40) and amyloid-beta 42 (Abeta42), major components of amyloid plaques, and the cytotoxic C-terminal fragments, gamma-CTF(50), gamma-CTF(57) and gamma-CTF(59). Many other minor beta-amyloid peptides, beta-amyloid 1-X peptides, are found in cerebral spinal fluid (CSF) including the beta-amyloid X-15 peptides, produced from the cleavage by alpha-secretase and all terminating at Gln-686. Proteolytically cleaved by caspases during neuronal apoptosis. Cleavage at Asp-739 by either caspase-6, -8 or -9 results in the production of the neurotoxic C31 peptide and the increased production of beta-amyloid peptides. N- and O-glycosylated. O-glycosylation on Ser and Thr residues with core 1 or possibly core 8 glycans. Partial tyrosine glycosylation (Tyr-681) is found on some minor, short beta-amyloid peptides (beta-amyloid 1-15, 1-16, 1-17, 1-18, 1-19 and 1-20) but not found on beta-amyloid 38, beta-amyloid 40 nor on beta-amyloid 42. Modification on a tyrosine is unusual and is more prevelant in AD patients. Glycans had Neu5AcHex(Neu5Ac)HexNAc-O-Tyr, Neu5AcNeu5AcHex(Neu5Ac)HexNAc-O-Tyr and O-AcNeu5AcNeu5AcHex(Neu5Ac)HexNAc-O-Tyr structures, where O-Ac is O-acetylation of Neu5Ac. Neu5AcNeu5Ac is most likely Neu5Ac 2,8Neu5Ac linked. O-glycosylations in the vicinity of the cleavage sites may influence the

proteolytic processing. Appicans are L-APP isoforms with O-linked chondroitin sulfate. Phosphorylation in the C-terminal on tyrosine, threonine and serine residues is neuron-specific. Phosphorylation can affect APP processing, neuronal differentiation and interaction with other proteins. Phosphorylated on Thr-743 in neuronal cells by Cdc5 kinase and Mapk10, in dividing cells by Cdc2 kinase in a cell-cycle dependent manner with maximal levels at the G2/M phase and, in vitro, by GSK-3-beta. The Thr-743 phosphorylated form causes a conformational change which reduces binding of Fe65 family members. Phosphorylation on Tyr-757 is required for SHC binding. Phosphorylated in the extracellular domain by casein kinases on both soluble and membrane-bound APP. This phosphorylation is inhibited by heparin.
Extracellular binding and reduction of copper, results in a corresponding oxidation of Cys-144 and Cys-158, and the formation of a disulfide bond. In vitro, the APP-Cu(+) complex in the presence of hydrogen peroxide results in an increased production of beta-amyloid-containing peptides. Trophic-factor deprivation triggers the cleavage of surface APP by beta-secretase to release sAPP-beta which is further cleaved to release an N-terminal fragment of APP (N-APP). Beta-amyloid peptides are degraded by IDE.

Cellular localization

Membrane. Membrane, clathrin-coated pit. Cell surface protein that rapidly becomes internalized via clathrin-coated pits. During maturation, the immature APP (N-glycosylated in the endoplasmic reticulum) moves to the Golgi complex where complete maturation occurs (O-glycosylated and sulfated). After alpha-secretase cleavage, soluble APP is released into the extracellular space and the C-terminal is internalized to endosomes and lysosomes. Some APP accumulates in secretory transport vesicles leaving the late Golgi compartment and returns to the cell surface. Gamma-CTF(59) peptide is located to both the cytoplasm and nuclei of neurons. It can be translocated to the nucleus through association with APBB1 (Fe65). Beta-APP42 associates with FRPL1 at the cell surface and the complex is then rapidly internalized. APP sorts to the basolateral surface in epithelial cells. During neuronal differentiation, the Thr-743 phosphorylated form is located mainly in growth cones, moderately in neurites and sparingly in the cell body. Casein kinase phosphorylation can occur either at the cell surface or within a post-Golgi compartment. Associates with GPC1 in perinuclear compartments. Colocalizes with SORL1 in a vesicular pattern in cytoplasm and perinuclear regions.

Images



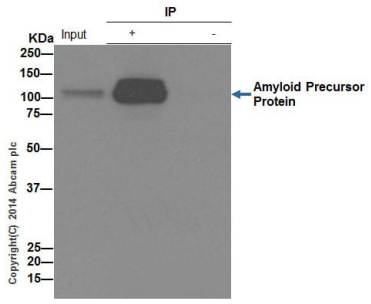
Western blot - Anti-Amyloid Precursor Protein antibody [EPR5119(2)] (ab133588)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: Amyloid Precursor Protein knockout HAP1 whole cell lysate (20 µg)
Lane 3: HepG2 whole cell lysate (20 µg)
Lane 4: HeLa whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab133588 observed at 110 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab133588 was shown to recognize Amyloid Precursor Protein when Amyloid Precursor Protein knockout samples were used, along with additional cross-reactive bands. Wild-type and Amyloid Precursor Protein knockout samples were subjected to SDS-PAGE. ab133588 and **ab9484** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-

Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

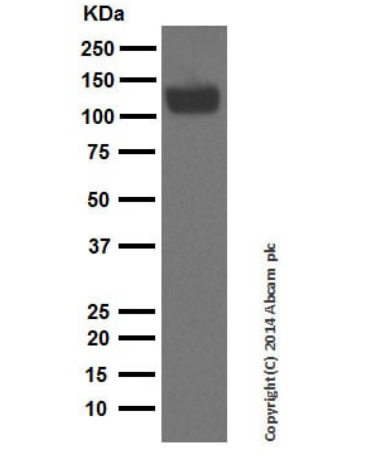


Immunoprecipitation - Anti-Amyloid Precursor Protein antibody [EPR5119(2)] (ab133588)

ab133588 (purified) at 1/20 immunoprecipitating amyloid precursor protein in human fetal brain (Lane 2). Lane 3 - PBS. For western blotting, a HRP-conjugated anti-rabbit IgG, specific to the non-reduced form of IgG was used as the secondary antibody (1/1500).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-Amyloid Precursor Protein antibody [EPR5119(2)] (ab133588)

Anti-Amyloid Precursor Protein antibody [EPR5119(2)] (ab133588) at 1/20000 dilution (purified) + Human fetal brain tissue lysate

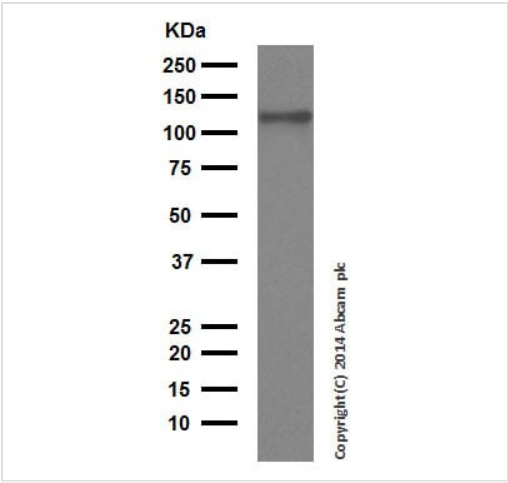
Secondary

HRP conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 86 kDa

Blocking Buffer: 5% NFDM/TBST

Dilution Buffer: 5% NFDM/TBST



Western blot - Anti-Amyloid Precursor Protein antibody [EPR5119(2)] (ab133588)

Anti-Amyloid Precursor Protein antibody [EPR5119(2)] (ab133588) at 1/2000 dilution (purified) + HEK-293 cell lysate at 20 µg

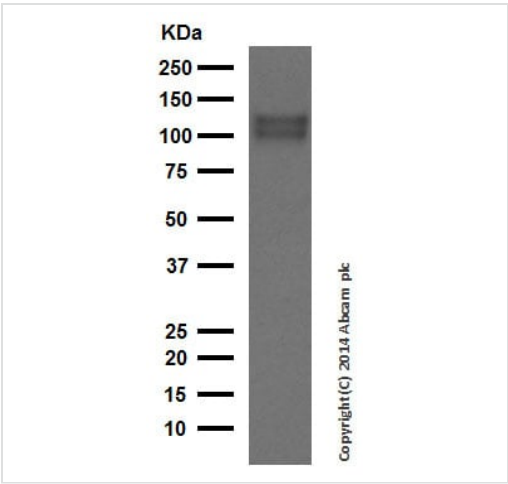
Secondary

HRP conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 86 kDa

Blocking Buffer: 5% NFDM/TBST

Dilution Buffer: 5% NFDM/TBST



Western blot - Anti-Amyloid Precursor Protein antibody [EPR5119(2)] (ab133588)

Anti-Amyloid Precursor Protein antibody [EPR5119(2)] (ab133588) at 1/2000 dilution (purified) + SH-SY5Y cell lysate at 20 µg

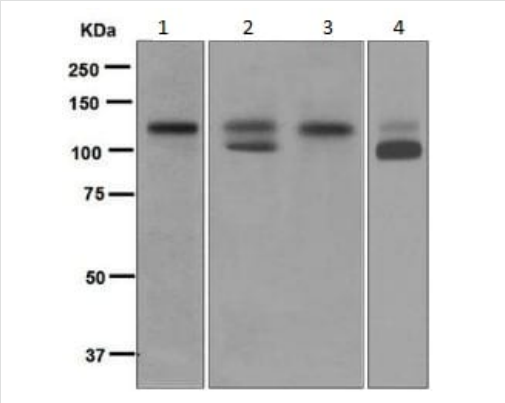
Secondary

HRP conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 86 kDa

Blocking Buffer: 5% NFDM/TBST

Dilution Buffer: 5% NFDM/TBST



Western blot - Anti-Amyloid Precursor Protein antibody [EPR5119(2)] (ab133588)

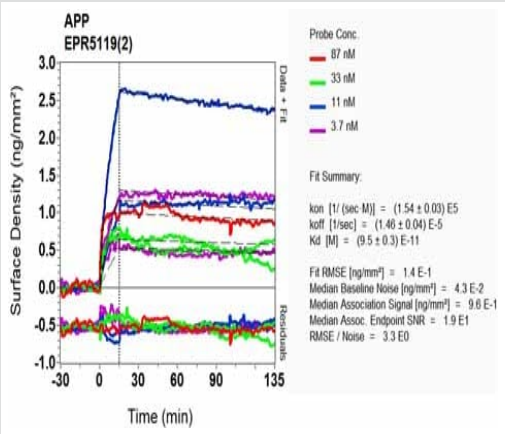
All lanes : Anti-Amyloid Precursor Protein antibody [EPR5119(2)] (ab133588) at 1/1000 dilution (unpurified)

Lane 1 : 293T cell lysate
Lane 2 : SH-SY5Y cell lysate
Lane 3 : U87 MG cell lysate
Lane 4 : Human fetal brain tissue lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP labelled Goat anti-Rabbit IgG at 1/2000 dilution

Predicted band size: 86 kDa
Observed band size: 100-120 kDa

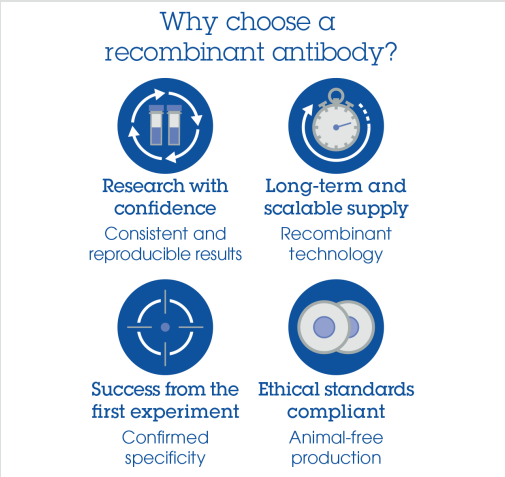


OI-RD Scanning - Anti-Amyloid Precursor Protein antibody [EPR5119(2)] (ab133588)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D



Anti-Amyloid Precursor Protein antibody

[EPR5119(2)] (ab133588)

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