abcam

Product datasheet

Anti-Amyloid Precursor Protein antibody [EPR5118] -BSA and Azide free ab248529





4 Images

Overview

Product name Anti-Amyloid Precursor Protein antibody [EPR5118] - BSA and Azide free

Description Rabbit monoclonal [EPR5118] to Amyloid Precursor Protein - BSA and Azide free

Host species Rabbit

Suitable for: WB **Tested applications**

Unsuitable for: Flow Cyt,ICC/IF,IHC-P or IP

Species reactivity Reacts with: Mouse, Human

Predicted to work with: Rat

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

General notes ab248529 is the carrier-free version of ab133509.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar[®] Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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**.

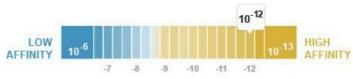
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Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Dissociation constant (K_D) $K_D = 1.80 \times 10^{-12} M$



Learn more about K_D

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

ClonalityMonoclonalClone numberEPR5118

Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab248529 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		Use at an assay dependent concentration. Predicted molecular weight: 86 kDa.

Application notes Is unsuitable for Flow Cyt,ICC/IF,IHC-P or IP.

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 BPTl/Kunitz inhibitor domain.

production of beta-amyloid peptides.

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

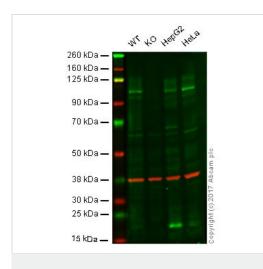
N- and O-glycosylated. O-glycosylation on Ser and Thr residues with core 1 or possibly core 8 glycans. Partial tyrosine glycosylation (Tyr-681) is found on some minor, short beta-amyloid peptides (beta-amyloid 1-15, 1-16, 1-17, 1-18, 1-19 and 1-20) but not found on beta-amyloid 38, beta-amyloid 40 nor 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	3
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Western blot - Anti-Amyloid Precursor Protein antibody [EPR5118] - BSA and Azide free (ab248529)

This data was developed using <u>ab133509</u>, the same antibody clone in a different buffer formulation.

Lane 1: Wild type HAP1 whole cell lysate (20 µg)

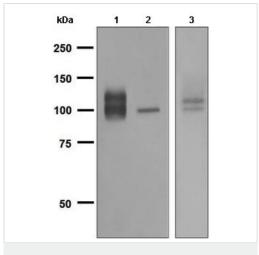
Lane 2: Amyloid Precursor Protein knockout HAP1 whole cell lysate (20 µg)

Lane 3: HepG2 whole cell lysate (20 µg)

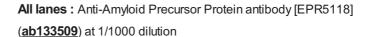
Lane 4: HeLa whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab133509</u> observed at 110 kDa. Red - loading control, <u>ab9484</u>, observed at 37 kDa.

ab133509 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. ab133509 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 lgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Amyloid Precursor Protein antibody [EPR5118] - BSA and Azide free (ab248529)



Lane 1: Human brain lysate
Lane 2: Human heart lysate
Lane 3: SHSY-5Y lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes: Goat-anti-rabbit HRP at 1/2000 dilution

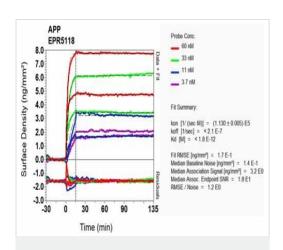
Predicted band size: 86 kDa

This data was developed using <u>ab133509</u>, the same antibody clone in a different buffer formulation.

This data was developed using $\underline{ab133509}$, the same antibody clone in a different buffer formulation. Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D



Ol-RD Scanning - Anti-Amyloid Precursor Protein antibody [EPR5118] - BSA and Azide free (ab248529)



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