## Overview

<table>
<thead>
<tr>
<th>Product name</th>
<th>Anti-Amyloid Precursor Protein antibody [Y188]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>Rabbit monoclonal [Y188] to Amyloid Precursor Protein</td>
</tr>
<tr>
<td>Host species</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Tested applications</td>
<td>Suitable for: WB, IHC-P, Flow Cyt, IP, ICC/IF</td>
</tr>
<tr>
<td>Species reactivity</td>
<td>Reacts with: Mouse, Rat, Human</td>
</tr>
<tr>
<td>Immunogen</td>
<td>Synthetic peptide (the amino acid sequence is considered to be commercially sensitive) within Human Amyloid Precursor Protein aa 750 to the C-terminus. The exact sequence is proprietary.</td>
</tr>
<tr>
<td>General notes</td>
<td>The immunogen used for this product is within Human Amyloid Precursor Protein aa 750 to the C-terminus and therefore may detect gamma secretase fragments 50, 57 and 59 in addition to fragments C31, C80, C83 and C99. Cross-reactivity with these fragments has not been confirmed experimentally.</td>
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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.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.
Form: Liquid

Storage instructions: Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

Storage buffer:
- pH: 7.20
- Preservative: 0.01% Sodium azide
- Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity: Protein A purified

Clonality: Monoclonal

Clone number: Y188

Isotype: IgG

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...

Properties

Applications

Our Abpromise guarantee covers the use of ab32136 in the following tested applications.

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

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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 prevalent 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 2,8 Neu5Ac 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**
All lanes: Anti-Amyloid Precursor Protein antibody [Y188] (ab32136) at 1/20000 dilution (purified)

Lane 1: HeLa cell lysate
Lane 2: Human fetal brain tissue lysate
Lane 3: HEK293 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes: HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 87 kDa
Observed band size: 95 kDa

why is the actual band size different from the predicted?

Blocking buffer: 5% NFDM/TBST
Dilution buffer: 5% NFDM/TBST
Immunohistochemical staining of paraffin embedded human glioblastoma with purified ab32136 at a working dilution of 1/500. The secondary antibody used is ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L), at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

Immunofluorescence staining of SH-SY5Y cells with purified ab32136 at a working dilution of 1 in 100, counter-stained with DAPI. Tubulin was stained with mouse anti-tubulin at a dilution of 1/1000 (ab7291) and Alexa Fluor® 594 goat anti-mouse at a dilution of 1/500 (ab150120). The secondary antibody was ab150077 Alexa Fluor® 488 goat anti-rabbit, used at a dilution of 1 in 500. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in the bottom middle and right hand panels - for the first negative control, purified ab32136 was used at a dilution of 1/200 followed by an Alexa Fluor® 555 goat anti-mouse antibody at a dilution of 1/500 and for the second negative control mouse primary antibody (ab7291) and anti-rabbit secondary antibody (ab15007) were used.
ab32136 (purified) at 1/30 immunoprecipitating amyloid beta precursor protein in A431 (Lane 1). Lane 2 - 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.

Overlay histogram showing PC-12 cells fixed in 2% PFA and stained with purified ab32136 at a dilution of 1 in 70 (red line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 150. Rabbit monoclonal IgG was used as an isotype control (black) and cells without antibody were used as a negative control (blue).

Unpurified ab32136, at a 1/250 dilution, staining Amyloid beta precursor protein by immunohistochemistry.

Positive immunohistochemical staining, using paraffin embedded human brain tissue (A).

Negative immunohistochemical staining, using human breast (B), skeletal muscle (C) and liver (D) tissues.

Tissues were stained in parallel on the same Normal Tissue Array.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
All lanes: Anti-Amyloid Precursor Protein antibody [Y188] (ab32136) at 1/20000 dilution (purified)

Lane 1: Mouse brain tissue lysate
Lane 2: Rat brain tissue lysate

Lysates/proteins at 20 µg per lane.

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All lanes: HRP goat anti-rabbit (H+L) at 1/1000 dilution

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Blocking buffer: 5% NFDM/TBST
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Anti-Amyloid Precursor Protein antibody [Y188] (ab32136) at 1/20000 dilution (unpurified) + Hela cell lysate

Predicted band size: 87 kDa
Observed band size: 95 kDa

Why is the actual band size different from the predicted?

Additional bands at: 110 kDa. We are unsure as to the identity of these extra bands.

Anti-Amyloid Precursor Protein antibody [Y188] (ab32136) at 1/1000 dilution (unpurified) + Mouse spleen whole cell lysate at 100 µg

Secondary
HRP-conjugated goat anti-rabbit polyclonal IgG at 1/4000 dilution

Developed using the ECL technique.
Performed under reducing conditions.

**Predicted band size:** 87 kDa

**Observed band size:** 95 kDa  *why is the actual band size different from the predicted?*

**Additional bands at:** 125 kDa. We are unsure as to the identity of these extra bands.

**Exposure time:** 10 minutes

Blocked with 5% milk

Overlay histogram showing PC12 cells stained with ab32136 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32136, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10⁶ cells) used under the same conditions. Unlabelled sample (blue line).

Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in PC12 cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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- Extensive multi-media technical resources to help you
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