abcam

Product datasheet

Anti-Amyloid Precursor Protein antibody [Y188] ab32136

KO 評価済 リコンビナント RabMAb

★★★★★ 8 Abreviews 216 References 画像数 11

製品の概要

製品名	Anti-Amyloid Precursor Protein antibody [Y188]
製品の詳細	Rabbit monoclonal [Y188] to Amyloid Precursor Protein
由来種	Rabbit
アプリケーション	**適用あり:** WB, IHC-P, IP, ICC/IF **適用なし:** Flow Cyt
種交差性	**交差種:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: HeLa, HAP1, U-87 MG and HEK-293 cell lysates; human hippocampus and fetal brain lysate; mouse brain lysate; rat brain lysate. IP: A431 cell lysate. IHC-P: Human brain tissue. ICC/IF: HeLa and SH-SY5Y cells.
特記事項	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. 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**.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
バッファー	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	Y188
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、次のテスト済みアプリケーションにおけるab32136の使用に適用されます**

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB	★★★★★ (4)	1/20000. Detects a band of approximately 95 kDa (predicted molecular weight: 87 kDa). **For unpurified, use 1/100 - 1/10000.**
IHC-P		1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
IP		1/30.
ICC/IF		1/100 - 1/500.

追加情報 Is unsuitable for Flow Cyt.

ターゲット情報

機能 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).

組織特異性

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.

関連疾患

Alzheimer disease 1
Cerebral amyloid angiopathy, APP-related

配列類似性

Belongs to the APP family.
Contains 1 BPTI/Kunitz inhibitor domain.

ドメイン

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.

翻訳後修飾

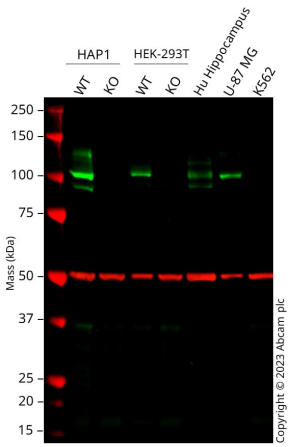
Proteolytically processed under normal cellular conditions. Cleavage either by alpha-secretase, beta-secretase or theta-secretase leads to generation and extracellular release of soluble APP peptides, S-APP-alpha and S-APP-beta, and the retention of corresponding membrane-anchored C-terminal fragments, 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.

細胞内局在

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.

画像



Western blot - Anti-Amyloid Precursor Protein antibody [Y188] (ab32136)

All lanes : Anti-Amyloid Precursor Protein antibody [Y188] (ab32136) at 1/20000 dilution

Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : APP knockout HAP1 cell lysate
Lane 3 : Wild-type HEK-293T cell lysate
Lane 4 : APP knockout HEK-293T cell lysate
Lane 5 : Human Hippocampus cell lysate
Lane 6 : U-87 MG cell lysate
Lane 7 : K562 cell lysate

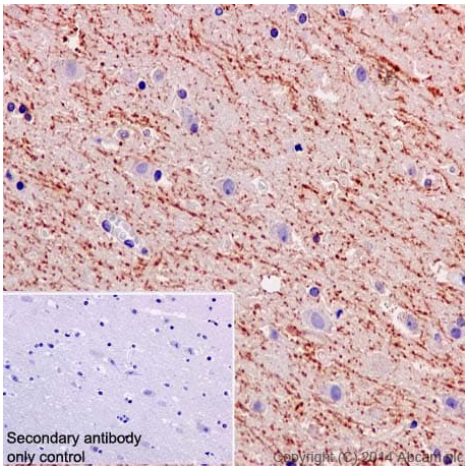
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 87 kDa

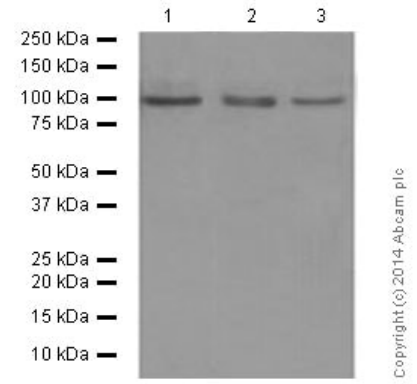
Observed band size: 80-130 kDa

Western blot: Anti-APP antibody [Y188] (ab32136) staining at 1/20000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32136 was shown to bind specifically to APP. A band was observed at 80-130 kDa in wild-type HAP1 and HEK-293T cell lysates with no signal observed at this size in both APP knockout cell lines. To generate this image, wild-type and APP knockout HAP1 and HEK-293T cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5% milk in TBS-0.1% Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Amyloid Precursor Protein antibody [Y188] (ab32136)

Immunohistochemical staining of paraffin embedded human gliocytoma 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 perfomed 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.



Western blot - Anti-Amyloid Precursor Protein antibody [Y188] (ab32136)

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

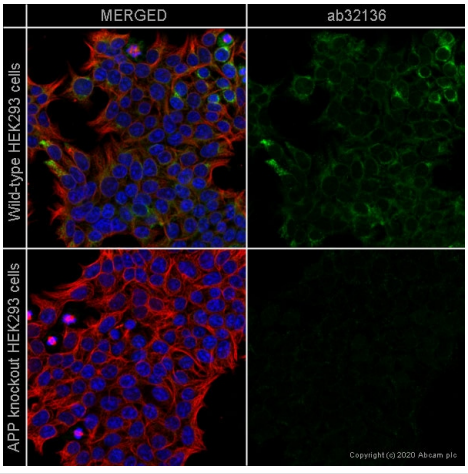
Lysates/proteins at 20 µg per lane.

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

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

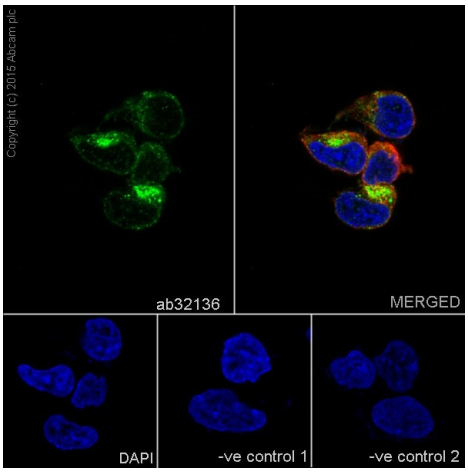
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



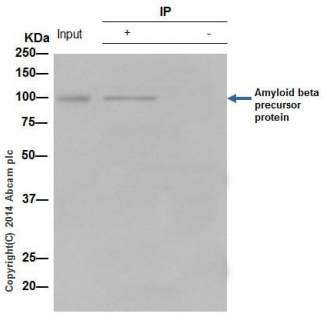
Immunocytochemistry/ Immunofluorescence - Anti-Amyloid Precursor Protein antibody [Y188] (ab32136)

ab32136 staining Amyloid Precursor Protein in wild-type HEK293 cells (top panel) and APP knockout HEK293 cells (**ab255362**) (bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab32136 at 1/500 dilution and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



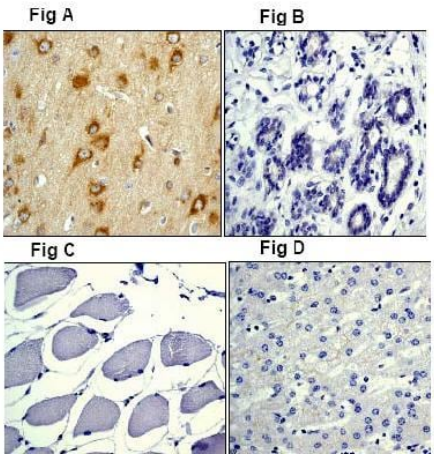
Immunocytochemistry/ Immunofluorescence - Anti-Amyloid Precursor Protein antibody [Y188] (ab32136)

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.



Immunoprecipitation - Anti-Amyloid Precursor Protein antibody [Y188] (ab32136)

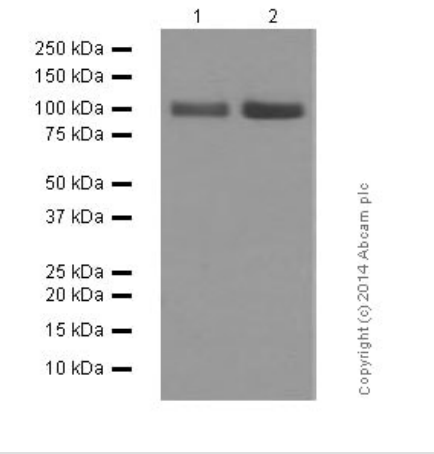
ab32136 (purified) at 1/30 immunoprecipitating amyloid beta precursor protein in A431 (Lane 1). Lane 2 - PBS. For western blotting a HRP-conjugated anti-rabbit IgG specific to the non-reduced form of IgG was used as the secondary antibody (1/1500). Blocking buffer and concentration: 5% NFDM/TBST. Diluting buffer and concentration: 5% NFDM /TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Amyloid Precursor Protein antibody [Y188] (ab32136)

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.



Western blot - Anti-Amyloid Precursor Protein antibody [Y188] (ab32136)

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

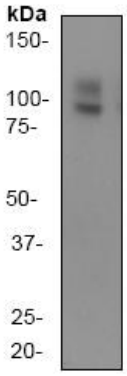
Lysates/proteins at 20 µg per lane.

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

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

Blocking buffer: 5% NFDM/TBST

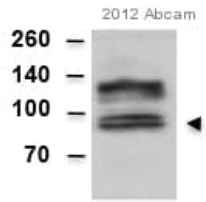
Dilution buffer: 5% NFDM/TBST



Western blot - Anti-Amyloid Precursor Protein antibody [Y188] (ab32136)

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



Western blot - Anti-Amyloid Precursor Protein antibody [Y188] (ab32136)
This image is courtesy of an anonymous Abreview

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

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

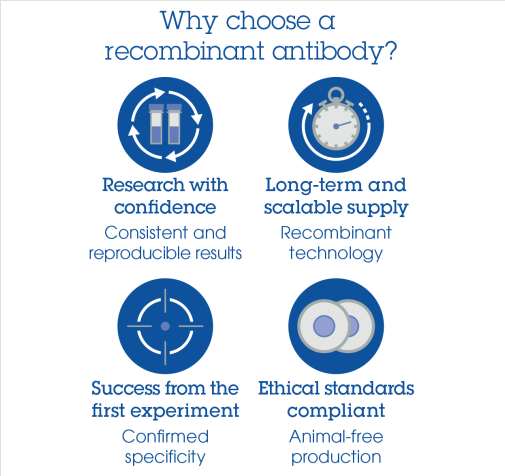
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 87 kDa
Observed band size: 95 kDa
Additional bands at: 125 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 10 minutes

Blocked with 5% milk



Anti-Amyloid Precursor Protein antibody [Y188]

(ab32136)

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