abcam

Product datasheet

Anti-BACE1 antibody [EPR19523] ab183612



★★★★★ 9 Abreviews 28 References 画像数 14

製品の概要

製品名 Anti-BACE1 antibody [EPR19523]

製品の詳細 Rabbit monoclonal [EPR19523] to BACE1

由来種 Rabbit

アプリケーション 適用あり: IHC-P, WB, IP, IHC-Fr

種交差性 交差種: Mouse, Rat, Human

免疫原 Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール WB: Mouse and rat hippocampus and brain lysates. IHC-P: Mouse hippocampus and cerebrum

tissues; rat cerebrum tissue. IHC-Fr: Mouse hippocampus tissue. IP: Rat and mouse

hippocampus whole cell lysates.

特記事項 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. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

バッファー pH: 7.2

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 EPR19523

アプリケーション

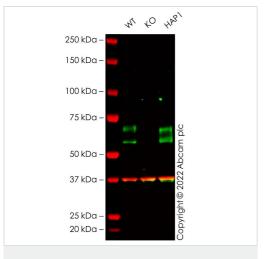
The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab183612の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
IHC-P	****(8)	1/50. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. IHC-P is recommended for mouse only. Binding in rat is weak under our experimental conditions and requires further optimization.
WB	★★★★ (1)	1/1000. Detects a band of approximately 68 kDa (predicted molecular weight: 56 kDa).
IP		1/50.
IHC-Fr		1/250. IHC-Fr is recommended for mouse only.

ターゲット情報

機能	Responsible for the proteolytic processing of the amyloid precursor protein (APP). Cleaves at the N-terminus of the A-beta peptide sequence, between residues 671 and 672 of APP, leads to the generation and extracellular release of beta-cleaved soluble APP, and a corresponding cell-associated C-terminal fragment which is later released by gamma-secretase.
組織特異性	Expressed at high levels in the brain and pancreas. In the brain, expression is highest in the substantia nigra, locus coruleus and medulla oblongata.
配列類似性	Belongs to the peptidase A1 family.
ドメイン	The transmembrane domain is necessary for its activity. It determines its late Golgi localization and access to its substrate, APP.
翻訳後修飾	Glycosylated.
細胞内局在	Membrane. Golgi apparatus > trans-Golgi network. Endoplasmic reticulum. Endosome. Cell surface. Predominantly localized to the later Golgi/trans-Golgi network (TGN) and minimally detectable in the early Golgi compartments. A small portion is also found in the endoplasmic reticulum, endosomes and on the cell surface.

画像



Western blot - Anti-BACE1 antibody [EPR19523] (ab183612)

All lanes : Anti-BACE1 antibody [EPR19523] (ab183612) at 1/1000 dilution

Lane 1: Wild-type SH-SY5Y cell lysate

Lane 2: Bace1 knockout SH-SY5Y cell lysate

Lane 3: HAP1 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

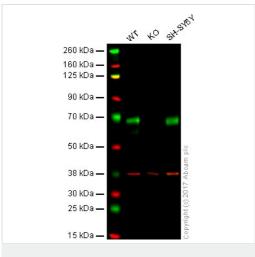
Predicted band size: 56 kDa **Observed band size:** 60,70 kDa

False colour image of Western blot: Anti-BACE1 antibody [EPR19523] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red.

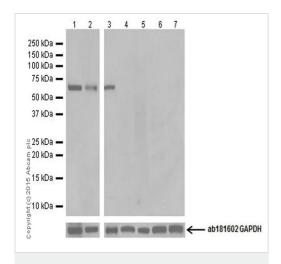
In Western blot, ab183612 was shown to bind specifically to BACE1. A band was observed at 60/70 kDa in wild-type SH-SY5Y cell lysates with no signal observed at this size in Bace1 knockout cell line <u>ab280078</u> (knockout cell lysate <u>ab280137</u>).

To generate this image, wild-type and Bace1 knockout SH-SY5Y cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane.

Membranes were blocked in 3 % 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 lgG H&L 800CW and Goat anti-Mouse lgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-BACE1 antibody [EPR19523] (ab183612)



Western blot - Anti-BACE1 antibody [EPR19523] (ab183612)

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

Lane 2: BACE1 knockout HAP1 whole cell lysate (20 µg)

Lane 3: SHSY5Y whole cell lysate (20 µg)

Lanes 1 - 3: Merged signal (red and green). Green - ab183612 observed at 68 kDa. Red - loading control, **ab181602**, observed at 37 kDa.

ab183612 was shown to specifically react with BACE1 in wild-type HAP1 cells as signal was lost in BACE1 knockout cells. Wild-type and BACE1 knockout samples were subjected to SDS-PAGE. ab183612 and <u>ab181602</u> (Rabbit anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed <u>ab216773</u> and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed <u>ab216776</u> secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

All lanes : Anti-BACE1 antibody [EPR19523] (ab183612) at 1/1000 dilution

Lane 1: Mouse hippocampus lysate

Lane 2: Rat brain lysate

Lane 3: Rat hippocampus lysate

Lane 4: Mouse ovary lysate

Lane 5 : Neuro-2a (Mouse neuroblastoma cell line) whole cell

lysate

Lane 6: C6 (Rat glial tumor cell line) whole cell lysate

Lane 7: SH-SY5Y (Human neuroblastoma cell line from bone

marrow) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

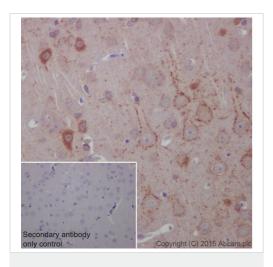
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 56 kDa **Observed band size:** 68 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1 and 2: 1 minute; Lane 3,4,5,6,7 and 8: 3

minutes.



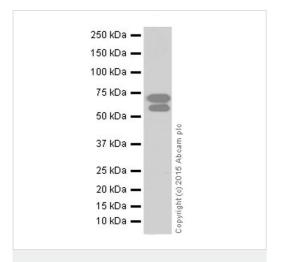
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BACE1 antibody
[EPR19523] (ab183612)

Immunohistochemical analysis of paraffin-embedded rat cerebrum tissue labeling BACE1 with ab183612 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasm staining on some neurons of the rat cerebrum is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Binding in rat was weak under our experimental conditions and requires further optimization.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-BACE1 antibody [EPR19523] (ab183612)

Anti-BACE1 antibody [EPR19523] (ab183612) at 1/1000 dilution + Mouse brain lysate at 10 μg

Secondary

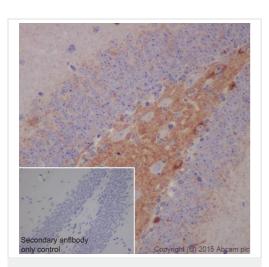
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 56 kDa **Observed band size:** 68 kDa

Exposure time: 30 seconds

An additional band was observed at 70 kD. The expression profile is consistent with what has been described in the literature (PMID: 22741101).

Blocking/Dilution buffer: 5% NFDM/TBST.

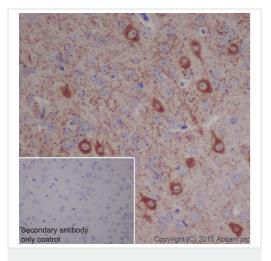


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BACE1 antibody
[EPR19523] (ab183612)

Immunohistochemical analysis of paraffin-embedded mouse hippocampus tissue labeling BACE1 with ab183612 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasm staining on mouse Hilar region of the dentate gyrus is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

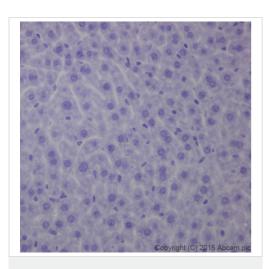


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BACE1 antibody
[EPR19523] (ab183612)

Immunohistochemical analysis of paraffin-embedded mouse cerebrum tissue labeling BACE1 with ab183612 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasm staining on neurons of the mouse cerebrum is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

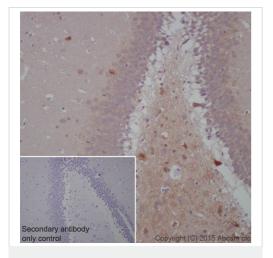


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BACE1 antibody
[EPR19523] (ab183612)

Immunohistochemical analysis of paraffin-embedded mouse liver tissue labeling BACE1 with ab183612 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Negative staining on mouse liver. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab97051** at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

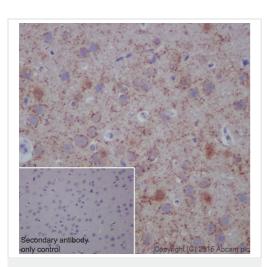


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BACE1 antibody
[EPR19523] (ab183612)

Immunohistochemical analysis of paraffin-embedded rat hippocampus tissue labelling BACE1 with ab183612 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. The sample was counterstained with hematoxylin. Antigen retrieval was performed using Tris/EDTA buffer; pH 9.0.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Binding in rat was weak under our experimental conditions and requires further optimization.

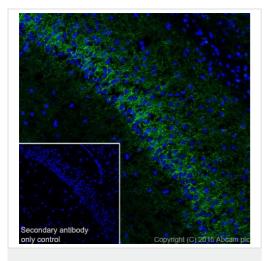


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BACE1 antibody
[EPR19523] (ab183612)

Immunohistochemical analysis of paraffin-embedded rat cerebrum tissue labelling BACE1 with ab183612 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. The sample was counterstained with hematoxylin. Antigen retrieval was performed using Tris/EDTA buffer; pH 9.0.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

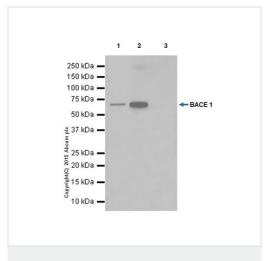
Binding in rat was weak under our experimental conditions and requires further optimization.



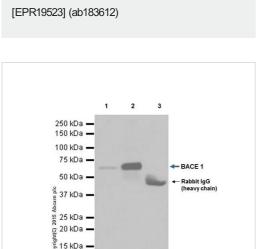
Immunohistochemistry (Frozen sections) - Anti-BACE1 antibody [EPR19523] (ab183612)

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen mouse hippocampus tissue labeling BACE1 with ab183612 at 1/250 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). The result showed mainly cytoplasmic staining on mouse hippocampus. The nuclear counterstain is DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit lgG (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution.



Immunoprecipitation - Anti-BACE1 antibody



Immunoprecipitation - Anti-BACE1 antibody [EPR19523] (ab183612)

10 kDa

BACE1 was immunoprecipitated from 1mg of rat hippocampus whole cell lysate with ab183612 at 1/50 dilution. Western blot was performed from the immunoprecipitate using ab183612 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

Lane 1: Rat hippocampus whole cell lysate, 10µg (Input).

Lane 2: ab183612 IP in Rat hippocampus whole cell lysate.

Lane 3: Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) instead of ab183612 in rat hippocampus whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 5 seconds.

BACE1 was immunoprecipitated from 1mg of mouse hippocampus whole cell lysate with ab183612 at 1/50 dilution. Western blot was performed from the immunoprecipitate using ab183612 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

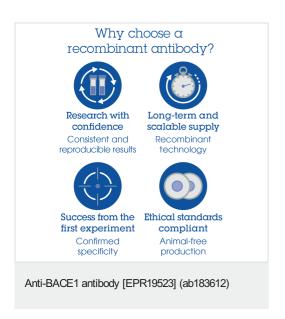
Lane 1: Mouse hippocampus whole cell lysate, 10µg (Input).

Lane 2: ab183612 IP in mouse hippocampus whole cell lysate.

Lane 3: Rabbit lgG,monoclonal [EPR25A] - Isotype Control (ab172730) instead of ab183612 in Mouse hippocampus whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 1 second.



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