# abcam

## Product datasheet

# Anti-BACE1 antibody [EPR19523] - BSA and Azide free ab238937



Recombinant

RabMAb

### 12 Images

#### Overview

Product name Anti-BACE1 antibody [EPR19523] - BSA and Azide free

**Description** Rabbit monoclonal [EPR19523] to BACE1 - BSA and Azide free

Host species Rabbit

**Tested applications** Suitable for: IHC-Fr, WB, IP, IHC-P

Species reactivity Reacts with: Mouse, Rat, Human

**Immunogen** Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

**Positive control** WB: HAP1 and SH-SY5Y cells.

**General notes** ab238937 is the carrier-free version of **ab183612**.

Our <u>carrier-free</u> 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 cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our <u>conjugation kits</u> 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<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> 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<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **patents**.

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#### **Properties**

Form Liquid

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

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

ClonalityMonoclonalClone numberEPR19523

**Isotype** IgG

#### **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab238937 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
IHC-Fr		Use at an assay dependent concentration.  IHC-Fr is recommended for mouse only.
WB		Use at an assay dependent concentration. Detects a band of approximately 68 kDa (predicted molecular weight: 56 kDa).
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. 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.

T	a	n	a	et

**Function** 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.

**Tissue specificity** Expressed at high levels in the brain and pancreas. In the brain, expression is highest in the

substantia nigra, locus coruleus and medulla oblongata.

**Sequence similarities** Belongs to the peptidase A1 family.

**Domain** The transmembrane domain is necessary for its activity. It determines its late Golgi localization

and access to its substrate, APP.

Post-translational

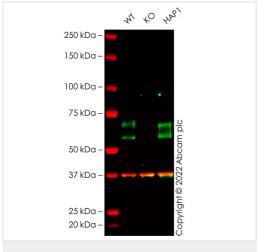
modifications

Glycosylated.

#### **Cellular localization**

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.

#### **Images**



Western blot - Anti-BACE1 antibody [EPR19523] - BSA and Azide free (ab238937)

**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, <u>ab183612</u> 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.

This data was developed using the same antibody clone in a different buffer formulation (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 - <u>ab183612</u> observed at 68 kDa. Red - loading control, <u>ab181602</u>, observed at 37 kDa.

<u>ab183612</u> 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. <u>ab183612</u> 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 IgG H&L (IRDye<sup>®</sup> 800CW) preabsorbed <u>ab216773</u> and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed <u>ab216776</u> secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

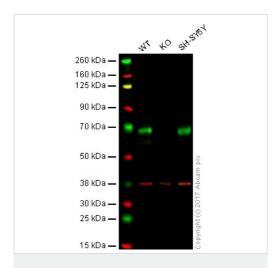
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab183612</u>).

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

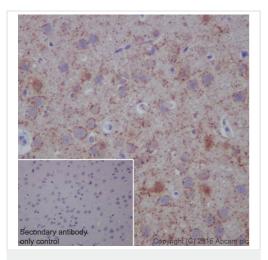
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

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

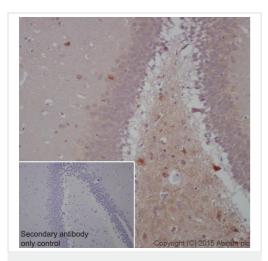
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab183612).



Western blot - Anti-BACE1 antibody [EPR19523] - BSA and Azide free (ab238937)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BACE1 antibody
[EPR19523] - BSA and Azide free (ab238937)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BACE1 antibody

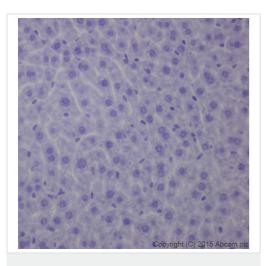
[EPR19523] - BSA and Azide free (ab238937)

Immunohistochemical analysis of paraffin-embedded rat hippocampus tissue labelling BACE1 with <u>ab183612</u> at 1/50 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab183612).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-BACE1 antibody

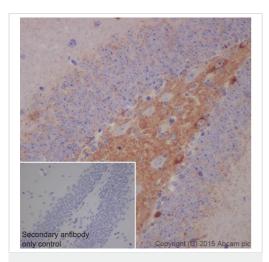
[EPR19523] - BSA and Azide free (ab238937)

Immunohistochemical analysis of paraffin-embedded mouse liver tissue labeling BACE1 with <u>ab183612</u> at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab183612).

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

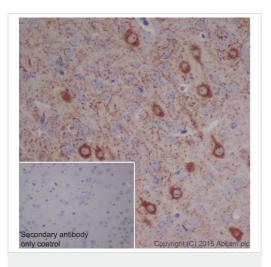
[EPR19523] - BSA and Azide free (ab238937)

Immunohistochemical analysis of paraffin-embedded mouse hippocampus tissue labeling BACE1 with <u>ab183612</u> at 1/50 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab183612</u>).

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

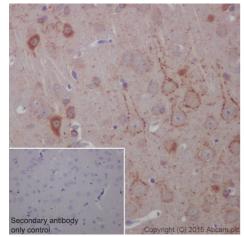
[EPR19523] - BSA and Azide free (ab238937)

Immunohistochemical analysis of paraffin-embedded mouse cerebrum tissue labeling BACE1 with <u>ab183612</u> at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab183612).

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] - BSA and Azide free (ab238937)

condary antibody

Immunohistochemistry (Frozen sections) - Anti-BACE1 antibody [EPR19523] - BSA and Azide free (ab238937)

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 IgG H&L (HRP) (ab97051) at 1/500 dilution.

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

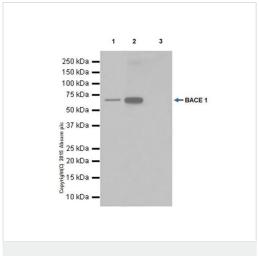
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab183612).

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

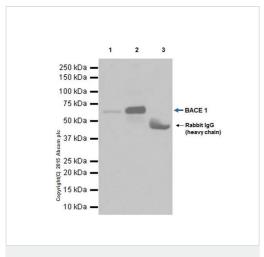
Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen mouse hippocampus tissue labeling BACE1 with ab183612 at 1/250 dilution, followed by Goat Anti-Rabbit lgG (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution (green). The result 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab183612</u>).



Immunoprecipitation - Anti-BACE1 antibody
[EPR19523] - BSA and Azide free (ab238937)



Immunoprecipitation - Anti-BACE1 antibody [EPR19523] - BSA and Azide free (ab238937) BACE1 was immunoprecipitated from 1mg of rat hippocampus whole cell lysate with <u>ab183612</u> at 1/50 dilution. Western blot was performed from the immunoprecipitate using <u>ab183612</u> at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), 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  $\lg G$ ,monoclonal [EPR25A] - lsotype Control  $(\underline{ab172730})$  instead of  $\underline{ab183612}$  in rat hippocampus whole cell lysate.

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

Exposure time: 5 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab183612</u>).

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

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

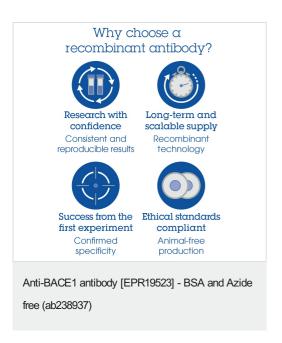
Lane 2: ab183612 IP in mouse hippocampus whole cell lysate.

Lane 3: Rabbit IgG,monoclonal [EPR25A] - Isotype
Control (<u>ab172730</u>) instead of <u>ab183612</u> in Mouse hippocampus whole cell Iysate.

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

Exposure time: 1 second.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab183612).



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