abcam

ab224267

Anti-Choline Acetyltransferase antibody [EPR13024(B)] - BSA and Azide free

Recombinant | RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR13024(B)
Purification technique	Affinity purification Protein A
Specificity	Some of our customers have successfully used ab181023 in mouse and rat. However, our latest tests were unsuccessful and therefore we can no longer guarantee the reactivity of these species. We recommend ab178850 as an alternative product for mouse and rat.
Concentration	1.04 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Transfected cell lysate - Human, Human
Dilution info	-
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Transfected cell lysate - Human, Mouse, Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Transfected cell lysate - Human, Mouse, Rat
Dilution info	-

Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Not recommended

Species	Transfected cell lysate - Human, Mouse, Rat
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	**For unpurified format use at 1/100 dilution.** Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Mouse, Rat, Transfected cell lysate - Human
Dilution info	-
Notes	-

IHC-Fr

Tested

Species	Human

Dilution info	-
Notes	-

Not recommended

Species	Transfected cell lysate - Human, Mouse, Rat
Dilution info	-
Notes	-

Target data

See full target information CHAT

Function	Catalyzes the reversible synthesis of acetylcholine (ACh) from acetyl CoA and choline at cholinergic synapses.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab224267 is the carrier-free version of ab181023.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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 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.

Compatibility
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.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Choline Acetyltransferase (ChAT) also known as EC 2.3.1.6 acts as the enzyme responsible for synthesizing the neurotransmitter acetylcholine. It catalyzes the transfer of an acetyl group from acetyl-CoA to choline to form acetylcholine. ChAT is a protein with an approximate mass of 70 kDa and is expressed in cholinergic neurons throughout the central and peripheral nervous systems. High expression is observed in regions such as the basal forebrain and neuromuscular junctions which are important for cholinergic signaling.
Biological function summary	The enzyme plays an important role in cholinergic conduction by producing acetylcholine which functions in synaptic transmission. It does not function as part of a larger complex but its activity directly influences cholinergic synapses. Choline acetyltransferase function ensures the synthesis and availability of acetylcholine for release into the synaptic cleft initiating muscle contraction and modulating activity in the central nervous system.
Pathways	Choline acetyltransferase is critical in pathways governing neurotransmitter release such as the cholinergic synapse pathway. It interacts functionally with other proteins within this pathway including acetylcholinesterase which breaks down acetylcholine in the synaptic cleft. This interaction ensures proper neurotransmitter regulation and recycling maintaining synapse function and communication within neuronal circuits.
Associated diseases and disorders	Alterations in choline acetyltransferase activity relate to neurological disorders including Alzheimer's disease and myasthenia gravis. In Alzheimer's disease reduced choline acetyltransferase function leads to decreased acetylcholine synthesis contributing to the cognitive decline observed in patients. In myasthenia gravis acetylcholine receptors are impaired but effective choline acetyltransferase activity is still necessary to manage neurotransmitter levels. A potential therapeutic angle involves targeting related proteins that exacerbate these conditions to support acetylcholine levels.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

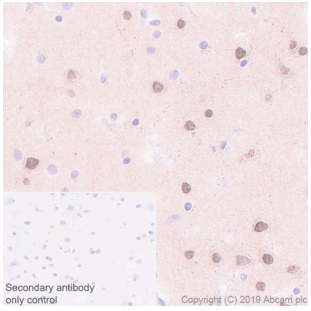
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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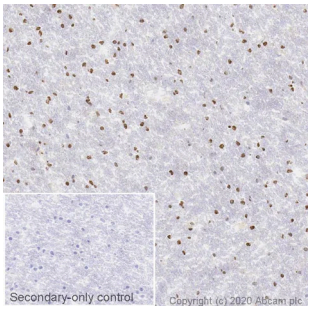
10 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Choline Acetyltransferase antibody [EPR13024(B)] - BSA and Azide free (ab224267)

Choline Acetyltransferase Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Choline Acetyltransferase antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human cerebrum tissue sections labeling Choline Acetyltransferase with purified ab181023 at 1/2000 dilution (0.28 µg/ml). Heat mediated antigen retrieval was performed using heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab181023).

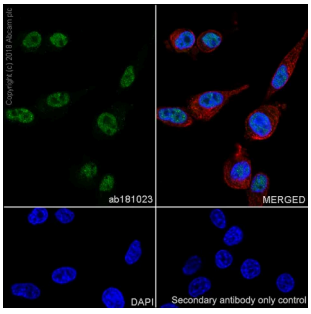


Immunohistochemistry (Frozen sections) - Anti-Choline Acetyltransferase antibody [EPR13024(B)] - BSA and Azide free (ab224267)

Choline Acetyltransferase Immunohistochemistry (Frozen sections) staining of normal human cerebral cortex using rabbit Anti-Choline Acetyltransferase antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab181023).
IHC image of Choline Acetyltransferase staining in a section of frozen normal human cerebral cortex performed on a Leica BOND™ system using the standard protocol. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab181023, 1/2000 dilution, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

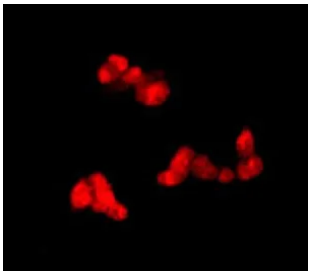
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunocytochemistry/ Immunofluorescence - Anti-Choline Acetyltransferase antibody [EPR13024(B)] - BSA and Azide free (ab224267)

Choline Acetyltransferase Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Choline Acetyltransferase antibody

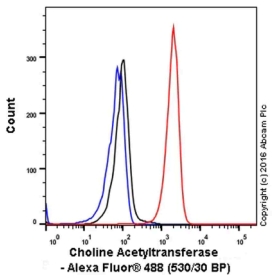
Immunocytochemistry/ Immunofluorescence analysis of U-87 MG (Human glioblastoma-astrocytoma epithelial cell) cells labeling Choline Acetyltransferase with purified ab181023 at 1/50 dilution (10 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1/1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab181023).



Immunocytochemistry/ Immunofluorescence - Anti-Choline Acetyltransferase antibody [EPR13024(B)] - BSA and Azide free (ab224267)

Choline Acetyltransferase Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Choline Acetyltransferase antibody

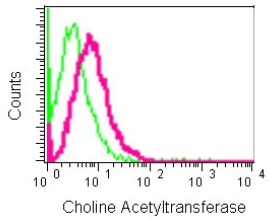
This ICC data was generated using the same anti-Choline Acetyltransferase antibody clone [EPR12024(B)] in a different buffer formulation (cat# ab181023).
Immunofluorescent analysis of 4% paraformaldehyde fixed SH-SY5Y cells labeling Choline Acetyltransferase using ab181023 (unpurified) at a 1/100 dilution. A Goat anti rabbit IgG (Alexa Fluor®555) was used as the secondary at a 1/100 dilution.



Flow Cytometry (Intracellular) - Anti-Choline Acetyltransferase antibody [EPR13024(B)] - BSA and Azide free (ab224267)

Choline Acetyltransferase Flow Cytometry (Intracellular) staining using rabbit Anti-Choline Acetyltransferase antibody

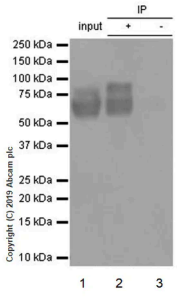
Intracellular Flow Cytometry analysis of SH-SY5Y (Human neuroblastoma epithelial cell) cells labeling Choline Acetyltransferase with purified ab181023 at 1/60 dilution (10µg/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab181023).



Flow Cytometry (Intracellular) - Anti-Choline Acetyltransferase antibody [EPR13024(B)] - BSA and Azide free (ab224267)

Choline Acetyltransferase Flow Cytometry (Intracellular) staining using rabbit Anti-Choline Acetyltransferase antibody

Intracellular Flow Cytometry analysis of SH-SY5Y cells labeling Choline Acetyltransferase using ab181023 (unpurified) at a 1/110 dilution (pink). Goat anti rabbit IgG (FITC) used as the secondary at a 1/150 dilution. Isotype control Rabbit monoclonal IgG (green). Cells were fixed in 2% paraformaldehyde.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab181023).



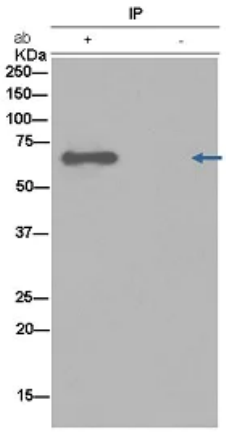
Immunoprecipitation - Anti-Choline Acetyltransferase antibody [EPR13024(B)] - BSA and Azide free (ab224267)

ab181023 (purified) at 1/30 dilution (2ug) immunoprecipitating Choline Acetyltransferase in Human fetal brain lysate. Human fetal brain lysate 10ug
Lane 2 (+): ab181023 & Human fetal brain lysate
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab181023 in Human fetal brain lysate
For western blotting, VeriBlot for IP secondary antibody (HRP) (ab131366) was used at 1/1000 dilution.
Blocking and diluting buffer: 5% NFDM/TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab181023).

All lanes:
Immunoprecipitation - Anti-Choline Acetyltransferase antibody [EPR13024(B)] (ab181023)

Predicted band size: 82 kDa

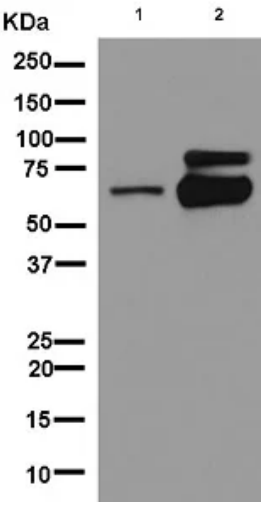


Immunoprecipitation - Anti-Choline Acetyltransferase antibody [EPR13024(B)] - BSA and Azide free (ab224267)

Lysate from Human fetal brain (Lane 1) and negative control (Lane 2) were immunoprecipitated with ab181023 (unpurified) at a 1/70 dilution. A anti-rabbit IgG (HRP), specific to the non-reduced form of IgG at a 1/1500 dilution for the secondary. Blocking/ Dilution buffer: 5% NFDM/TBST.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab181023).

All lanes:
Immunoprecipitation - Anti-Choline Acetyltransferase antibody [EPR13024(B)] (ab181023)

Predicted band size: 82 kDa



Western blot - Anti-Choline Acetyltransferase antibody [EPR13024(B)] - BSA and Azide free (ab224267)

Choline Acetyltransferase Western blot staining using rabbit Anti-Choline Acetyltransferase antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab181023).
Blocking/ Dilution buffer: 5% NFDM /TBST.

All lanes:
Western blot - Anti-Choline Acetyltransferase antibody [EPR13024(B)] (ab181023) at 1/5000 dilution

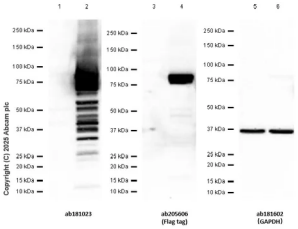
Lane 1:
SH-SY5Y cell lysate at 20 µg

Lane 2:
Human fetal brain lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 82 kDa



Western blot - Anti-Choline Acetyltransferase antibody [EPR13024(B)] - BSA and Azide free (ab224267)

Choline Acetyltransferase Western blot staining using rabbit Anti-Choline Acetyltransferase antibody

This data was developed using ab181023, the same antibody clone in a different buffer formulation.
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/1,000,000 dilution.

Lanes 1 - 2:

Western blot - Anti-Choline Acetyltransferase antibody [EPR13024(B)] (ab181023) at 1/1000 dilution

Lanes 1 - 4:
Western blot - Anti-DDDDK tag (Binds to FLAG® tag sequence) antibody [EPR20018-251] (ab205606) at 1/1000 dilution

Lanes 1 and 3:
293T cells transfected with an empty vector containing a flag tag whole cell lysate at 20 µg

Lanes 2 and 4:
293T cells transfected with a full-length human ChAT expression vector containing a flag-tag whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 82 kDa, 36 kDa

Exposure time: 3s

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.