abcam

Product datasheet

Anti-Choline Acetyltransferase antibody [EPR13024(B)] ab181023



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Overview

Product name Anti-Choline Acetyltransferase antibody [EPR13024(B)]

Description Rabbit monoclonal [EPR13024(B)] to Choline Acetyltransferase

Host species Rabbit

Specificity The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P,

Flow Cyt (Intra), IHC-Fr, ICC/IF and IP for mouse and rat.

Tested applications Suitable for: IHC-P, Flow Cyt (Intra), IHC-Fr, ICC/IF, IP, WB

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: SH-SY5Y and C6 cell lysate, human fetal brain lysate, mouse and rat brain lysates IP: Human

fetal brain lysate Flow Cyt (intra): SH-SY5Y cells ICC/IF: U-87 MG and SH-SY5Y cells IHC-P:

Human cerebrum tissue IHC-Fr: Hu cerebral cortex tissue sections.

General notesThis 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**.

Properties

Form Liquid

Storage instructions 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.

Storage buffer Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

1

Clonality Monoclonal

Clone number EPR13024(B)

Isotype IgG

Applications

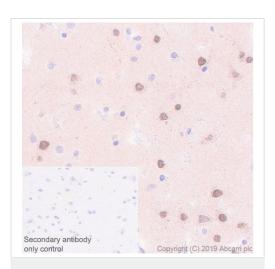
The Abpromise guarantee Our Abpromise guarantee covers the use of ab181023 in the following tested applications.

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

Application	Abreviews	Notes
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See IHC antigen retrieval protocols .
		For unpurified format use at 1/100 dilution.
		The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.
Flow Cyt (Intra)		1/60 - 1/110. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
IHC-Fr	★★★★ <u>(1)</u>	Use at an assay dependent concentration.
ICC/IF		1/50 - 1/100.
IP		1/30 - 1/70.
WB		1/1000 - 1/10000. Predicted molecular weight: 82 kDa.

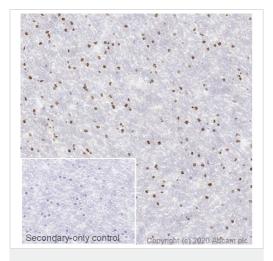
Target		
Function	Catalyzes the reversible synthesis of acetylcholine (ACh) from acetyl CoA and choline at cholinergic synapses.	
Involvement in disease	Myasthenic syndrome, congenital, 6, presynaptic	
Sequence similarities	Belongs to the carnitine/choline acetyltransferase family.	

Images



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Choline Acetyltransferase antibody [EPR13024(B)] (ab181023)

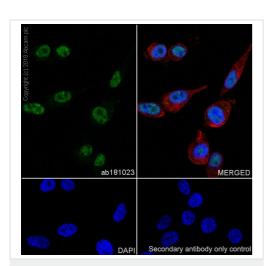
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human cerebrum tissue sections labeling Choline Acetyltransferase with purified ab181023 at 1/2000 dilution (0.28 µg/ml). Heat mediated antigen retrieval was performed using heat mediated antigen retrieval using ab93684 (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.



Immunohistochemistry (Frozen sections) - Anti-Choline Acetyltransferase antibody [EPR13024(B)] (ab181023)

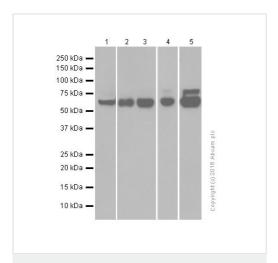
IHC image of Choline Acetyltransferase staining in a section of frozen normal human cerebral cortex performed on a Leica BONDTM system using the standard protocol. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab181023, 1/2000 dilution, for 15 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)] (ab181023)

Immunocytochemistry/ Immunofluorescence analysis of U-87 MG (Human glioblastoma-astrocytoma epithelial cell) cells labeling Choline Acetyltransferase with purified ab181023 at 1/50 dilution (10 μ g/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with <u>ab195889</u> Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) at 1:200 (2.5 μ g/ml). Goat anti rabbit lgG (Alexa Fluor[®] 488, <u>ab150077</u>) was used as the secondary antibody at 1/1000 (2 μ g/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Western blot - Anti-Choline Acetyltransferase antibody [EPR13024(B)] (ab181023)

All lanes : Anti-Choline Acetyltransferase antibody [EPR13024(B)] (ab181023) at 1/2000 dilution (Purified)

Lane 1 : SH-SY5Y (Human neuroblastoma epithelial cell) whole cell lysates

Lane 2: Mouse brain lysates

Lane 3: Rat brain lysates

Lane 4: C6 (Rat glial tumor glial cell) whole cell lysates

Lane 5: Human fetal brain lysates

Lysates/proteins at 20 µg per lane.

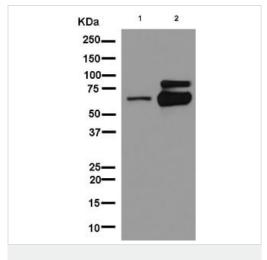
Secondary

 $\begin{tabular}{l} \textbf{All lanes:} Goat Anti-Rabbit \ lgG (HRP) with minimal cross-reactivity with human \ lgG \ at 1/2000 \ dilution \end{tabular}$

Predicted band size: 82 kDa **Observed band size:** 70-82 kDa

This antibody recognizes all Choline Acetyltransferase isoforms

with the MW ranging from 70-82 KDa based on immunogen blast.



Western blot - Anti-Choline Acetyltransferase antibody [EPR13024(B)] (ab181023)

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

Lane 1: SH-SY5Y cell lysate

Lane 2: Human fetal brain lysate

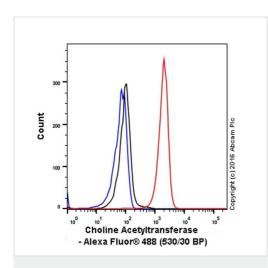
Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 82 kDa

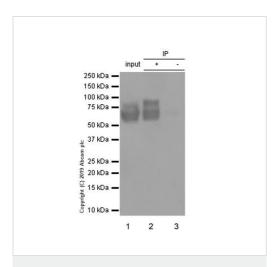
Blocking/ Dilution buffer: 5% NFDM /TBST.



Flow Cytometry (Intracellular) - Anti-Choline Acetyltransferase antibody [EPR13024(B)] (ab181023)

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 lgG (Alexa Fluor[®] 488, <u>ab150077</u>) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal lgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



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

ab181023 (purified) at 1/30 dilution (2ug) immunoprecipitating Choline Acetyltransferase in Human fetal brain lysate. Human fetal brain lysate 10ug

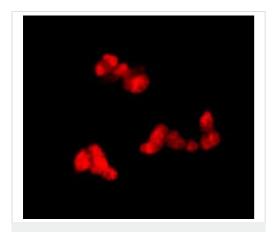
Lane 2 (+): ab181023 & Human fetal brain lysate
Lane 3 (-): Rabbit monoclonal lgG (ab172730) instead of

ab181023 in Human fetal brain lysate

For western blotting, VeriBlot for IP secondary antibody (HRP) $\,$

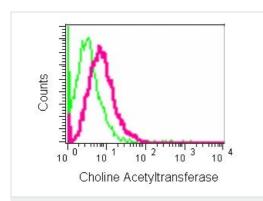
(<u>ab131366</u>) was used at 1/1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.



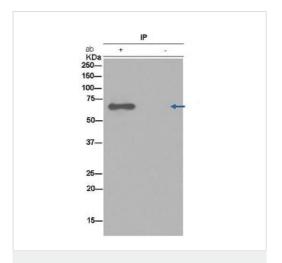
Immunocytochemistry/ Immunofluorescence - Anti-Choline Acetyltransferase antibody [EPR13024(B)] (ab181023)

Immunofluorescent analysis of 4% paraformaldehyde fixed SH-SY5Y cells labeling Choline Acetyltransferase using ab181023 (unpurified) at a 1/100 dilution. A Goat anti rabbit lgG (Alexa Fluor®555) was used as the secondary at a 1/100 dilution.



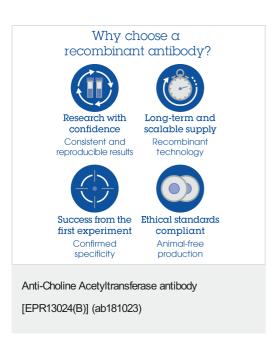
Flow Cytometry (Intracellular) - Anti-Choline Acetyltransferase antibody [EPR13024(B)] (ab181023)

Intracellular Flow Cytometry analysis of SH-SY5Y cells labeling Choline Acetyltransferase using ab181023 (unpurified) at a 1/110 dilution (pink). Goat anti rabbit lgG (FITC) used as the secondary at a 1/150 dilution. Isotype control Rabbit monoclonal lgG (green). Cells were fixed in 2% paraformaldehyde.



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

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 lgG (HRP), specific to the non-reduced form of lgG at a 1/1500 dilution for the secondary. Blocking/ Dilution buffer: 5% NFDM/TBST.



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