abcam

Product datasheet

Anti-Dopamine Transporter antibody [EPR19695] ab184451



12 References 13 Images

Overview

Product name Anti-Dopamine Transporter antibody [EPR19695]

Description Rabbit monoclonal [EPR19695] to Dopamine Transporter

Host species Rabbit

Specificity Human species is recommended based on IHC-P results, we do not guarantee IHC-Fr, WB and

IP for human.

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

Unsuitable for: ICC

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: Unboiled Mouse and rat striatum lysates. IHC-P: Mouse and rat striatum tissues, normal

human striatum, normal human substantia nigra. IHC-Fr: Mouse brain (Coronal section), rat brain

(sagittal section). IP: Mouse striatum whole cell lysate.

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 supplyAnimal-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 pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

1

Purity Protein A purified

Clonality Monoclonal
Clone number EPR19695

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab184451 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
WB		1/1000. Detects a band of approximately 70-85 kDa (predicted molecular weight: 69 kDa).
IHC-P		1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IHC-Fr		1/500.
IP		1/30.

Application notes Is unsuitable for ICC.

Target

Function Amine transporter. Terminates the action of dopamine by its high affinity sodium-dependent

reuptake into presynaptic terminals.

Involvement in disease Defects in SLC6A3 are the cause of dystonia-parkinsonism infantile (DYTPRI) [MIM:613135]. It is

a neurodegenerative disorder characterized by infantile onset of parkinsonism and dystonia.

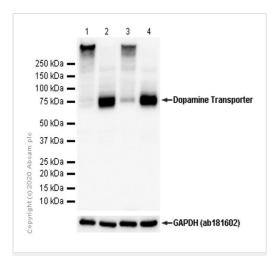
Other neurologic features include global developmental delay, bradikinesia and pyramidal tract

signs.

Sequence similaritiesBelongs to the sodium:neurotransmitter symporter (SNF) (TC 2.A.22) family. SLC6A3 subfamily.

Cellular localization Membrane.

Images



Western blot - Anti-Dopamine Transporter antibody [EPR19695] (ab184451)



Lane 1 : Mouse striatum lysate boiled

Lane 2: Mouse striatum lysate unboiled

Lane 3: Rat striatum lysate boiled

Lane 4: Rat striatum lysate unboiled

Lysates/proteins at 20 µg per lane.

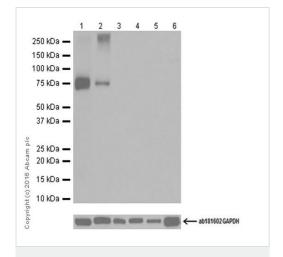
Secondary

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

Predicted band size: 69 kDa

We recommend not to boil the samples after lysis to get desired WB results.

ab181602 was used as a GAPDH loading control.



Western blot - Anti-Dopamine Transporter antibody [EPR19695] (ab184451)

All lanes : Anti-Dopamine Transporter antibody [EPR19695] (ab184451) at 1/1000 dilution

Lane 1: Mouse striatum lysate

Lane 2: Rat striatum lysate

Lane 3: Mouse kidney lysate

Lane 4: Rat liver lysate

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

lysate

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

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at

1/100000 dilution

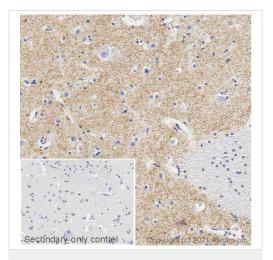
Predicted band size: 69 kDa **Observed band size:** 70-85 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

ab181602 was used as GAPDH loading control.

Dopamine transporters are distributed widely across the brain in regions with high dopaminergic activity, such as the striatum and substantia nigra (PMID:1765147), so non-related tissues and cells were chosen as negative controls; and DAT is a glycoprotein with molecular weight of 70-85KD (PMID:25356398, PMID: 20643191).



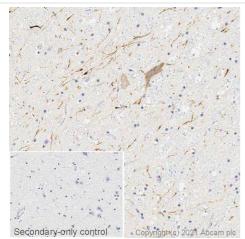
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Dopamine Transporter antibody [EPR19695] (ab184451)

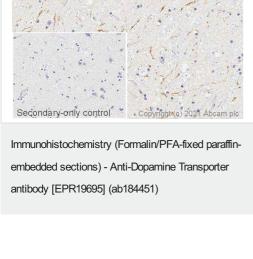
IHC image of Dopamine Transporter staining in a section of formalin fixed paraffin embedded normal human striatum* performed on a Leica BOND™. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab184451, 5ugml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen.

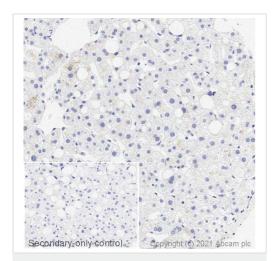
The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

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.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre







Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Dopamine Transporter antibody [EPR19695] (ab184451)

IHC image of Dopamine Transporter staining in a section of formalin fixed paraffin embedded normal human substantia nigra* performed on a Leica BOND™. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was incubated with ab184451, 5ugml, 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.

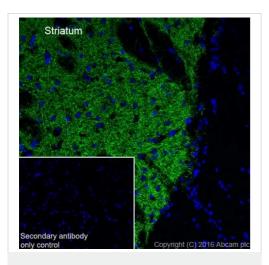
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

Negative control image: IHC image of Dopamine Transporter staining in a section of formalin fixed paraffin embedded normal human liver* performed on a Leica BOND™. The section was pretreated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins..The section was incubated with ab184451, 5ugml, 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.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



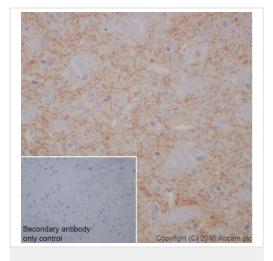
Immunohistochemistry (Frozen sections) - Anti-Dopamine Transporter antibody [EPR19695] (ab184451)

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen Rat brain (sagittal section) tissue labeling Dopamine Transporter with ab184451 at 1/500 dilution, followed by Goat Anti-Rabbit $\lg G$ (Alexa Fluor 488) (ab150077) secondary antibody at 1/1000 dilution (green).

The result showed cytoplasmic staining on rat striatum.

The nuclear counterstain is DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is <u>ab150077</u> at 1/1000 dilution.



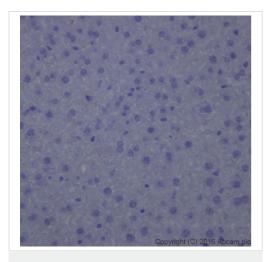
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Dopamine Transporter antibody [EPR19695] (ab184451)

Immunohistochemical analysis of paraffin-embedded Mouse striatum tissue labeling Dopamine Transporter with ab184451 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Membrane and cytoplasm staining on mouse striatum is observed. Counter stained with Hematoxylin.

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

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



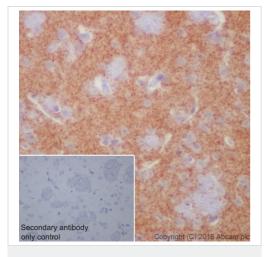
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Dopamine Transporter antibody [EPR19695] (ab184451)

Immunohistochemical analysis of paraffin-embedded Mouse liver tissue labeling Dopamine Transporter with ab184451 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Counter stained with Hematoxylin.

Negative control: Negative staining on mouse liver.

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-Dopamine Transporter antibody [EPR19695] (ab184451)

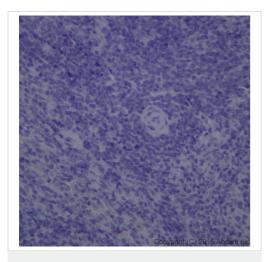
Immunohistochemical analysis of paraffin-embedded Rat striatum tissue labeling Dopamine Transporter with ab184451 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Cytoplasm staining on rat striatum is observed.

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.



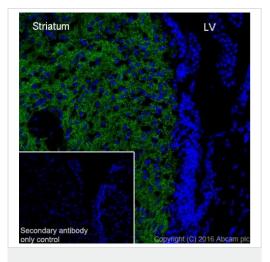
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Dopamine Transporter antibody [EPR19695] (ab184451)

Immunohistochemical analysis of paraffin-embedded Rat spleen tissue labeling Dopamine Transporter with ab184451 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Counter stained with Hematoxylin.

Negative control: Negative staining on rat spleen.

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

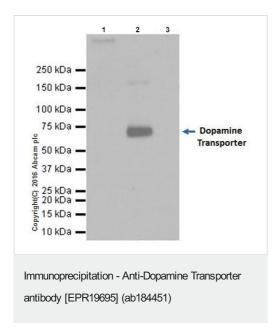


Immunohistochemistry (Frozen sections) - Anti-Dopamine Transporter antibody [EPR19695] (ab184451)

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen Mouse brain (Coronal section) tissue labeling Dopamine Transporter with ab184451 at 1/500 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green). The result showed cytoplasmic staining on mouse striatum but negative on lateral ventricle (LA).

The nuclear counterstain is DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is <u>ab150077</u> at 1/1000 dilution.



Dopamine Transporter was immunoprecipitated from 0.35mg of Mouse striatum whole cell lysate with ab184451 at 1/30 dilution.

Western blot was performed from the immunoprecipitate using ab184451 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10000 dilution.

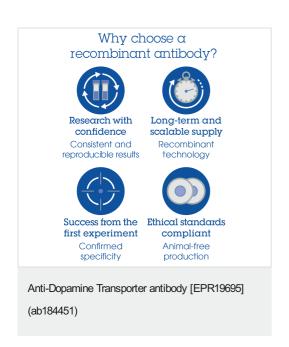
Lane 1: Mouse striatum whole cell lysate, 10µg (Input).

Lane 2: ab184451 IP in Mouse striatum whole cell lysate.

Lane 3: Rabbit IgG, monoclonal [EPR25A] - Isotype Control (<u>ab172730</u>) instead of ab184451 in Mouse striatum whole cell lysate.

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

Exposure time: 30 seconds.



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